UNIVERSIDAD COMPLUTENSE DE MADRID

FACULTAD DE ÓPTICA Y OPTOMETRÍA



TESIS DOCTORAL

Abordaje múltiple de la presbicia: corrección óptica e implicaciones moleculares

A multiple approach presbyopia: optical correction and molecular implications

MEMORIA PARA OPTAR AL GRADO DE DOCTORA

PRESENTADA POR

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"Los libros me enseñaron a pensar, y el pensamiento me hizo libre"

Ricardo León

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RESUMEN

Título

Abordaje múltiple de la presbicia: corrección óptica e implicaciones moleculares.

Introducción

La presbicia es una condición fisiológica que conlleva una pérdida progresiva de la capacidad de acomodación del ojo, dificultando las tareas próximas con el paso de los años. La corrección óptica multifocal, mediante lentes de contacto (LCs) y lentes intraoculares (LIOs) multifocales, es actualmente la opción más común para solventar los problemas visuales de la presbicia, demostrando altas tasas de independencia de gafas. En este contexto, la investigación en el ámbito de diseños para corregir la presbicia está en avance, con nuevos diseños para extender el rango de distancias en las que la visión sea nítida (extended depth-of-focus, EDOF).

En cuanto a las LCs, los factores principales que afectan al éxito de la adaptación son la visión y la comodidad durante el porte, los cuales son calificados como igual de importantes por los usuarios. Además, la integridad de la superficie ocular debería tenerse en cuenta antes, durante y después del proceso de adaptación, al igual que la repetibilidad de los dispositivos utilizados para su evaluación.

En cuanto a las LIOs para corregir la presbicia, su función visual y calidad óptica han sido ampliamente estudiadas. Sin embargo, ciertos factores, bien relativos al individuo (cirugías corneales previas) o al propio procedimiento quirúrgico (error refractivo residual, descentramiento de la LIO, rotación o inclinación) deben tenerse en cuenta a la hora de evaluar la calidad óptica, ya que podrían ser de gran ayuda en la práctica clínica para satisfacer las necesidades individuales de cada sujeto.

Por otra parte, la edad no solo es un factor clave en el comienzo de la presbicia, sino que es, a su vez, un promotor del síndrome de fragilidad y un factor determinante para las enfermedades asociadas a la edad. Este síndrome se describe como un estado de vulnerabilidad generalizada y de predisposición a la enfermedad causado por desequilibrios entre las citoquinas pro y antiinflamatorias, favoreciendo, así, una respuesta inflamatoria crónica. En este ámbito, el porte de LCs podría considerarse como un factor de riesgo externo, amplificando la cascada inflamatoria ya presente en la superficie ocular présbita como consecuencia de los mecanismos moleculares que subyacen al proceso de envejecimiento. Además, la determinación de biomarcadores de fragilidad podría ser clave para detectar este estado de forma temprana y evitar la aparición de las enfermedades oculares asociadas a la edad, así como identificar aquellos individuos en los que las LCs no se sean la opción más adecuada.

Objetivos y resultados

Tras un introductorio Capítulo 1, se presentan cinco estudios, una revisión bibliográfica y un artículo de opinión relacionados con estos temas. El Capítulo 2 se centra en el estudio de las LCs y LIOs para la corrección de la presbicia, mientras que el Capítulo 3 lo hace en las implicaciones moleculares de este periodo.

El Capítulo 2 tiene como objetivo evaluar la repetibilidad de las medidas tomadas con el dispositivo Keratograph 5M (K5M) en usuarios de LC para la posterior evaluación de los parámetros clínicos. Tras esto, se comparó la integridad de la superficie ocular tras el uso de una LC EDOF y un diseño asférico convencional, así como la función visual y la sintomatología. Los resultados mostraron niveles estables de repetibilidad para las medidas de altura de menisco lagrimal, tiempos de ruptura de la película lagrimal y enrojecimiento ocular al retirar la LC realizando tres medidas. La repetibilidad de la medida del tiempo medio de ruptura lagrimal se vio afectada por el factor “porte de LC”, pero no por el factor “tiempo de

porte". Además, las curvas de desenfoque, la función de sensibilidad al contraste, el rango de profundidad de foco, la percepción subjetiva de halo y deslumbramiento, y la sintomatología fueron similares entre el diseño EDOF y el diseño asférico de LC, así como su impacto en la superficie ocular.

Asimismo, se evaluó el efecto sobre la calidad óptica de diferentes situaciones halladas en la implantación de varios diseños de LIOs para corregir la presbicia. Para ello, se simularon en banco óptico y se analizaron mediante las curvas de función de transferencia de modulación las siguientes situaciones: desenfoque (error refractivo residual), rotaciones, inclinación, descentramiento y aberraciones esféricas positivas (ablaciones corneales miópicas). Los resultados mostraron una tolerancia de hasta 5° de rotación y 0.25D de desenfoque para LIOs trifocales tóricas tras evaluar dos potencias cilíndricas distintas (1.5D y 3.0D). Además, la calidad óptica de la LIO EDOF mostró más tolerancia frente a aberraciones esféricas positivas, mientras que el diseño trifocal mostró más tolerancia frente a inclinaciones y descentramientos.

Por otra parte, el Capítulo 3 se centra en las implicaciones moleculares de la presbicia en el sistema ocular. Para ello se analizó el efecto del uso de LC en la expresión de varios genes inflamatorios en la superficie ocular présbita. El inflamasoma NLRP3 y la mucina de membrana MUC16 mostraron una sobreexpresión en usuarios de LC en comparación con los no usuarios, mientras que la proteína CD73 mostró una expresión reducida. Esto sugiere que estos biomarcadores inflamatorios podrían ser un factor clave en la aparición de la fragilidad, promoviendo las enfermedades oculares asociadas a la edad.

Mediante búsqueda bibliográfica se halló que estos biomarcadores son moduladores de la migración de los neutrófilos y de su proceso de NETosis, y que los neutrófilos a su vez estaban asociados al proceso de fragilidad. Por esto motivo, se realizó una revisión bibliográfica sobre el papel de los neutrófilos y sus trampas extracelulares en la patogénesis de las enfermedades

oculares asociadas a la edad. La búsqueda mostró una relación entre estas dos variables, indicando que los neutrófilos estaban implicados en el desarrollo de estas enfermedades oculares asociadas a la edad.

Estos hechos sugieren que CD73 y NLRP3 podrían ser biomarcadores de fragilidad de la superficie ocular, y que mediante su capacidad para modular la migración de los neutrófilos y sus procesos de NETosis, podrían favorecer finalmente el desarrollo de enfermedades oculares asociadas a la edad. Teniendo en cuenta que la gran mayoría de présbitas acudirán al profesional de la salud visual para corregir los síntomas visuales de la presbicia, este momento que se enmarca en un rango de edad muy definido, podría aprovecharse para validar e identificar biomarcadores de fragilidad, y estudiar los mecanismos moleculares de la fragilidad a nivel ocular.

Conclusiones

Las principales conclusiones de esta tesis se encuentran resumidas en el Capítulo 4.

En lo relativo al estudio de la repetibilidad de las medidas tomadas con el dispositivo K5M, tres medidas son necesarias para obtener niveles de repetibilidad excelentes. Las LCs podrían considerarse un artefacto que afecta a la repetibilidad de la variable tiempo medio de ruptura de la película lagrimal, aunque esta no se ve afectada por el tiempo de porte. Además, la LC EDOF y el diseño asférico se comportan de manera similar en términos de rendimiento visual, sintomatología e integridad de la superficie ocular, aunque ambas LCs aumentan los síntomas de sequedad ocular.

En lo relativo al estudio de las LIOs, la lente trifocal tórica muestra una tolerancia de hasta 5° de rotación y 0.25D de desenfoque. Más allá de estos valores se produce un empeoramiento significativo de la calidad óptica. Además, la LIO EDOF muestra mejor tolerancia frente a aberraciones esféricas positivas, mientras que la LIO trifocal tolera mejor los descentramientos.

En cuanto al análisis molecular, el porte de LC blandas promueve una respuesta inflamatoria al modificar la expresión de ciertos biomarcadores inflamatorios de la superficie ocular présbita. Estos biomarcadores podrían considerarse biomarcadores de fragilidad, la cual predispone al desarrollo de enfermedades oculares asociadas a la edad. De esta forma, la presbicia podría tratarse como una oportunidad para el estudio y la detección temprana de estos biomarcadores, con el fin de evitar la aparición de las enfermedades asociadas a la edad.

Por último, a partir de los resultados obtenidos en esta tesis, se presentan futuras líneas de investigación, tanto relativas a la corrección óptica de la presbicia, como a las implicaciones moleculares del proceso de envejecimiento.

ABSTRACT

Title

A multiple approach to presbyopia: optical correction and molecular implications.

Introduction

Presbyopia is a physiological condition leading to a progressive decline of the ability of the eye to focus, thus making close-up work worsen over time. Multifocal optical correction, either by multifocal contact lenses (CLs) or intraocular lenses (IOLs), is currently the most common option to provide good vision at various distances, showing high rates of spectacle-independence. In this context, new designs are being developed aiming to extend the range of distances with satisfactory vision (extended depth of focus; EDOF).

Regarding CLs, the main factors affecting fitting success are vision and comfort, which are rated by wearers as equally important. Moreover, ocular surface integrity should also be taken into account before, during and after CL fitting process, along with the repeatability of the devices used for its evaluation.

Regarding presbyopia-correcting IOLs, their visual and optical performance has been widely assessed. However, several factors related to both the patient (previous surgeries) and the surgical procedure itself (residual refractive error, lens decentration, rotation and tilt) should be taken into consideration when evaluating IOL's optical quality, since they might be useful in clinical practice to meet the needs of each individual.

On the other hand, age is not only a key factor in presbyopia onset, but is also a promoter of frailty syndrome and a determinant factor for age-related diseases. This syndrome is described as a state of vulnerability and predisposition to disease caused by pro-inflammatory and anti-

inflammatory cytokine imbalances, favouring a chronic inflammatory response. In this context, long-term CL wear might act as an external factor amplifying the inflammatory cascade already present in the presbyopic ocular surface due to the underlying age-related molecular mechanisms. Furthermore, the determination of frailty biomarkers would allow early detection of this syndrome, avoiding the onset of age-related eye diseases, as well as predicting those individuals in which CLs to correct presbyopia are not recommended.

Objectives and results

After an introductory Chapter 1, five studies, one literature review and one opinion article regarding these topics are presented. Chapter 2 focuses on the assessment of CLs and IOLs to correct presbyopia, whereas Chapter 3 focuses on the molecular implications of this period of life on the ocular environment.

Chapter 2 aims to assess the repeatability of the Keratograph 5M (K5M) measurements in CL wearers before using it to assess clinical parameters. Afterwards, a comparison was performed between an EDOF design and a conventional aspheric CL regarding visual function, ocular surface integrity and symptomatology. The outcomes for K5M device showed stable repeatability for tear meniscus height (TMH), non-invasive keratograph break-up times (NIKBUTs) and ocular redness when the CL was removed from the eye and three measurements were performed. During CL wear, intrarater repeatability decreased for NIKBUT-average (average of all break-up times), but was not affected by the time of wear. Additionally, defocus curves, contrast sensitivity function, depth of focus range, halo and glare perception, and symptomatology were similar between the EDOF CL and the aspheric design, as well as their effect on the ocular surface.

On the other hand, the effect on the optical quality of different situations encountered when implanting presbyopia-correcting IOLs was assessed. Thus, defocus (residual refractive

errors), rotations, tilt, decentration, and positive spherical aberrations (previous corneal myopic ablations) were simulated in the optical bench and analysed via modulation transfer function (MTF) analysis.

The outcomes showed that there was some tolerance to rotation up to 5° and defocus up to 0.25D for trifocal toric IOLs when two different cylindric powers (1.5D and 3.0D) were evaluated. Moreover, in terms of optical quality, the presence of previous myopic ablation was better tolerated by EDOF design than by trifocal IOLs, whereas tilt and decentration were better tolerated by the trifocal design.

On the other hand, Chapter 3 aims to assess the molecular implications of presbyopia on the ocular environment. Accordingly, the effect of long-term CL wear on genes linked to inflammation on the presbyopic ocular surface was evaluated. NLRP3 inflammasome and membrane-bound mucin MUC16 were overexpressed in CL wearers compared no non-CL wearers, whereas CD73 protein showed a reduced expression. These findings suggest that these inflammation biomarkers could be a key factor in frailty onset, promoting age-related diseases. By a bibliographic search it was found that these biomarkers were modulators of neutrophil migration and the NETosis process, and that, at the same time, neutrophils were associated with the frailty process. Thus, a literature review was performed regarding the role of neutrophils and their extracellular traps on the pathogenesis of age-related eye diseases. The literature showed a link between these two variables, indicating that neutrophils are implicated in the development of age-related eye diseases. These facts suggest that CD73 and NRLP3 could be frailty biomarkers of the ocular surface and that they could favour age-related eye diseases onset by the modulation of neutrophil migration and NETosis processes.

Considering that most presbyopes will visit eye health professionals to correct their presbyopic visual symptoms, this moment that belongs to a defined age range could be used to validate and identify frailty biomarkers and to study the molecular mechanisms of ocular frailty.

Conclusions

The main conclusions of this work are summarised in Chapter 4. Regarding the study of the repeatability of K5M measurements, it can be stated that three measurements are recommended to obtain excellent results. CLs might be considered an artifact affecting NIKBUT-average results, but this parameter seems to not be affected by the time of wear. Additionally, the EDOF design and the aspheric CL perform similarly when assessing visual performance, ocular surface integrity and symptomatology, although both CLs increase dry eye symptoms. Regarding the study of presbyopia-correcting IOLs, trifocal toric IOLs present a tolerance to defocus up to 0.25D and to rotations up to 5°. Exceeding those limits significantly decreases optical quality. Moreover, EDOF IOLs tolerate positive spherical aberrations better than trifocal IOLs, whereas trifocal IOLs tolerate tilt and decentrations better than the EDOF design. Concerning the molecular analysis, it can be concluded that long-term soft CL wear seems to promote an inflammatory response by modifying the expression of certain inflammatory biomarkers on the ocular surface. These biomarkers could serve as frailty biomarkers, promoting age-related eye disease onset. In this way, presbyopia can be considered an outstanding opportunity for frailty biomarkers detection to avoid age-related disease onset.

Lastly, from the results obtained in this thesis, several future lines of research are suggested in Chapter 4, including the optical correction of presbyopia, as well as the molecular implications of the ageing process.

ABBREVIATIONS

- ADO: adenosine
- AGEs: advanced glycation end products
- AMD: age-related macular degeneration
- ARED: age-related eye disease
- ATP: adenosine triphosphate
- CCL: C-C Motif Chemokine Ligand
- CD: centre-distance
- cDNA: complementary DNA
- CH: conventional hydrogel
- CI: confidence interval
- CL: contact lens
- CLD: contact lens discomfort
- CLW: contact lens wearer
- CN: centre-near
- CoV: coefficient of variation
- CR: coefficient of repeatability
- CS: contrast sensitivity
- D: dioptre
- DD: daily disposable
- DED: dry eye disease
- DNA: deoxyribonucleic acid
- DOF: depth of focus
- DR: diabetic retinopathy

- EDOF: extended depth of focus
- EGF: epidermal growth factor
- ETDRS: early treatment diabetic retinopathy study
- GM-CSF: granulocyte-macrophage colony-stimulating factor
- ICC: intraclass correlation coefficient
- IFN: interferon
- IL: interleukin
- IOL: intraocular lens
- K5M: Keratograph 5M
- LASIK: laser in situ keratomileusis
- LCN: lipocalin
- logMAR: logarithm of the minimum angle of resolution
- LT: leukotriene
- MMP: metalloprotease
- MPO: myeloperoxidase
- mRNA: messenger RNA
- MTF: modulation transfer function
- MUC: mucin
- NBR: nasal bulbar redness
- NCLW: non-contact lens wearer
- NGF: nerve growth factor
- NLR: nasal limbal redness
- NLRP: NOD-like receptor protein
- NIKBUT-average: average of all non-invasive keratograph break-up times
- NIKBUT-first: first non-invasive keratograph break-up time

- NOD: nucleotide-binding and oligomerization domain
- oBRB: outer blood-retina barrier
- OSDI: Ocular Surface Disease Index
- PCR: polymerase chain reaction
- POAG: primary open-angle glaucoma
- qPCR: quantitative PCR
- RNA: ribonucleic acid
- SA: spherical aberration
- SD: standard deviation
- SiH: silicone hydrogel
- SII: systemic immune- inflammation index
- Sw: within-subject SD
- TBR: temporal bulbar redness
- TFOS: Tear Film and Ocular Surface Society
- TGF: transforming growth factor
- TIMP: tissue inhibitor of metalloproteinase
- TLR: temporal limbal redness
- TM: trabecular meshwork
- TMH: tear meniscus height
- TNF: tumour necrosis factor
- VA: visual acuity
- VEGF: vascular endothelial growth factor

LIST OF FIGURES

Figure 1. The light from the out-of-focus focal point (blurred image) creates a halo in the focused point (clear image).

Figure 2. Ray tracing representation of a single vision CL (A) and a multifocal CL (B). The single vision CL creates a single focal point, for which the optical quality is optimal, but progressively decreases out of this point. In the multifocal CLs, multiple focal points are created, elongating the depth of focus (DOF). The optical quality along this DOF is not optimal, but it is acceptable, providing multifocality.

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Figure 11. Diagram of the relation between age, inflammation and CL wear. Inflammation is a common pathway in all age, frailty and age-related diseases. CL have shown to induce the release of inflammatory molecules in the tear film, and are considered a risk factor for subjects suffering from frailty syndrome.

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Figure 13. Tear meniscus height measurement with the K5M device.

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Figure 16. Study protocol. Measurements performed in each visit. HVID: horizontal visible iris diameter; TMH: tear meniscus height; NIKBUT: non-invasive keratograph break-up time; NIKBUT-average: average of all break-up times; OSDI: ocular surface disease index questionnaire.

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CHAPTER 1.

Chapter 1 includes an explanation for the title of the thesis and an introduction regarding the state of the art of the optical alternatives to correct presbyopia and the molecular implications of ageing on the ocular surface.

Moreover, a justification for the production of this thesis and its objectives are presented herein.

WHY THIS TITLE?

Presbyopia is a physiological condition leading to a progressive decline of the ability of the eye to focus, thus making close-up work worsen over time. Its onset is around 40 years old, and it affects 100% of the population by age 50. Given the impact of this event on the individual's quality of life, the vast majority of the population will seek presbyopia correction and visit eye care professionals.

However, besides the clinical implications of age in the eye (presbyopia), attention should be paid to other ocular levels, such as the molecular level. Age is the main promoter of frailty syndromes and age-related diseases, including ocular diseases (e.g., dry eye, diabetic retinopathy, age-related macular degeneration, or glaucoma). This syndrome is nothing but a state of vulnerability and predisposition to disease, which facilitates age-related disease onset. Thus, early detection of this syndrome will prevent or, at least, delay its poor prognosis.

In this context, presbyopia can be considered an outstanding opportunity for frailty syndrome early detection, since a significant proportion of the presbyopic population will seek eye health care. Thus, presbyopia not only should be studied in the optical context, but also regarding the molecular implications in age-related diseases. This thesis will focus on this multiple approach to presbyopia.

INTRODUCTION

1.1.PRESBYOPIA

Presbyopia is a physiological and progressive decline in the amplitude of accommodation. Age-related changes such as hardening, thickening and loss of elasticity of the crystalline lens contribute to the loss of the focus ability, directly affecting near work (1). The onset of presbyopia becomes evident and partially incapacitating in the fourth decade of life, when the individual's requirements for near vision are no longer accomplished (2).

Globally, presbyopia is the leading cause of vision loss and its prevalence is estimated at around 1.8 billion people (3). In 2020, a total of 510 million people worldwide suffered from visual impairment because of uncorrected presbyopia, and due to the ageing population, it is estimated that in 2050 the total number will reach 866 million (4).

Undoubtedly, uncorrected presbyopia is associated with reduced quality of life in both developing and developed countries (5), and this decrease is comparable to that associated with hypertension (6). Additionally, presbyopia not only affects the quality of life and vision, but it is also associated with a significant burden when it is not corrected, mostly in developing countries and rural areas (3), which results, at the same time, in a productivity loss. It is estimated that the correction of presbyopia could reduce this burden by around 1,390 billion dollars (7). Correcting presbyopia is considered a strikingly cost-effective way of restoring individuals' daily activities (8) and a priority regarding their quality of life (9).

1.2. OPTICAL CORRECTION

1.2.1. SPECTACLES: READING, BIFOCALS, AND PROGRESSIVE LENSES

Within the methods to correct presbyopia, spectacles, either single vision, bifocal, or progressive lenses, are one of the most common non-surgical options among presbyopes (10). Despite providing acceptable vision, spectacles are still the source of several visual symptoms and complaints, such as limited peripheral vision, glasses dependence, difficulty doing physical, recreational and day-to-day activities (11), headaches, balance issues and nausea, among others (12).

Additionally, presbyopia corrected with glasses is related to a worsening in quality of life (6), and it has been shown that the quality of vision worsens with the amount of time using reading glasses (10). Therefore, presbyopes are seeking spectacle-free options to correct presbyopia, such as contact lenses or ocular surgery.

1.2.2. PRESBYOPIA-CORRECTING CONTACT LENSES

Contact lenses (CLs) are another option to correct presbyopia, by wearing CLs for distance with near glasses, monovision strategies or presbyopia-correcting designs. Although glasses for near vision over CLs for distance offer good vision, spectacle-dependence is the main drawback of this option. Monovision consists of fitting a CL for near in one eye, and for distance in the contralateral. This fitting option is truly pupil-independent, and therefore does not induce visual disturbances in dim light conditions or low contrast. However, the reduced stereoacuity is still the main limitation of this strategy (13–15), along with the inability of the binocular system to suppress blur from the defocused image (16). On the other hand,

presbyopia-correcting CLs were developed to provide good vision at different distances and reduce spectacle-dependence.

CL prescription rate varies among studies and nations. Currently, presbyopia-correcting CLs outperforms monovision, with fitting rates of 31% and 8%, respectively (17). Additionally, it is estimated that only 37% of the presbyopic population is fitted with a presbyopic prescription, with 29% wearing multifocal lenses and 8% fitted with monovision (17). Recent studies showed a low rate of 2.8% for both monovision and presbyopic lens fitting (10).

Due to the increasing presbyopic population, a rise in presbyopic fitting rates should be expected in the next years. However, despite improvements in CL options and designs, it seems to be a significant under-prescribing of presbyopia-correcting CLs. It is important to note that the prescription rate drastically decreases with age, mostly when reaching the age of 35-44, which coincides with the onset of presbyopia (17).

Several hypotheses are being considered for this under-prescribing of presbyopia-correcting CLs, among which is the lack of a "perfect" CL satisfying comfort during wear without compromising visual demands (17). In this context, presbyopes report vision and comfort as equally important reasons for CL dropout (18) and opt for CL correction when both factors can be successfully achieved (19). Therefore, vision and comfort are considered the main factors affecting CL fitting success.

CLs to correct presbyopia can be classified into alternating/translating vision or simultaneous image lenses. Alternating vision CLs are the lens version of bifocal spectacles, based on the direction of the gaze to change from distance to near correction. The distance correction zone is usually in the upper half of the lens, which is used in "straight-ahead" gaze, whereas the add power is placed in the lower half. On downgaze, the lower lid supports the lens so the add power is placed in front of the pupil. Translating lenses are usually

manufactured in rigid materials, and have several disadvantages, just like bifocals. They only work if the near object is in the appropriate gaze position, they tend to have problems with rotation and stability, and image jumping commonly occurs between the two distinct power zones (20,21). These facts leave simultaneous-image CLs at the forefront of presbyopic designs.

Simultaneous-image principle arises from more than one image focusing simultaneously on the retina coming from the different lens powers within the pupil. The focused image will be accompanied by a blurred image (see Figure 1). This concept is based on blur interpretation or tolerance, where the neural system chooses between the in-focus and the out-of-focus images (22,23).

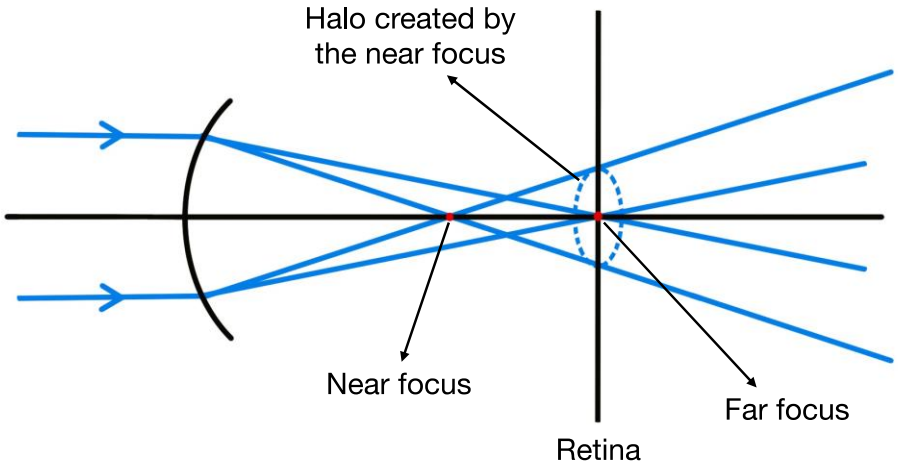


Figure 1. *The light from the out-of-focus focal point (blurred image) creates a halo in the focused point (clear image).*

Those several focal points across the retina will create a range of distances or focusing errors (depth of focus, DOF) that provide an acceptable and tolerable vision with a minimal difference from the in-focus image (see Figure 2). This concept relies on compensating the lack of multifocality in the presbyopic eye given by the loss of the amplitude of accommodation.

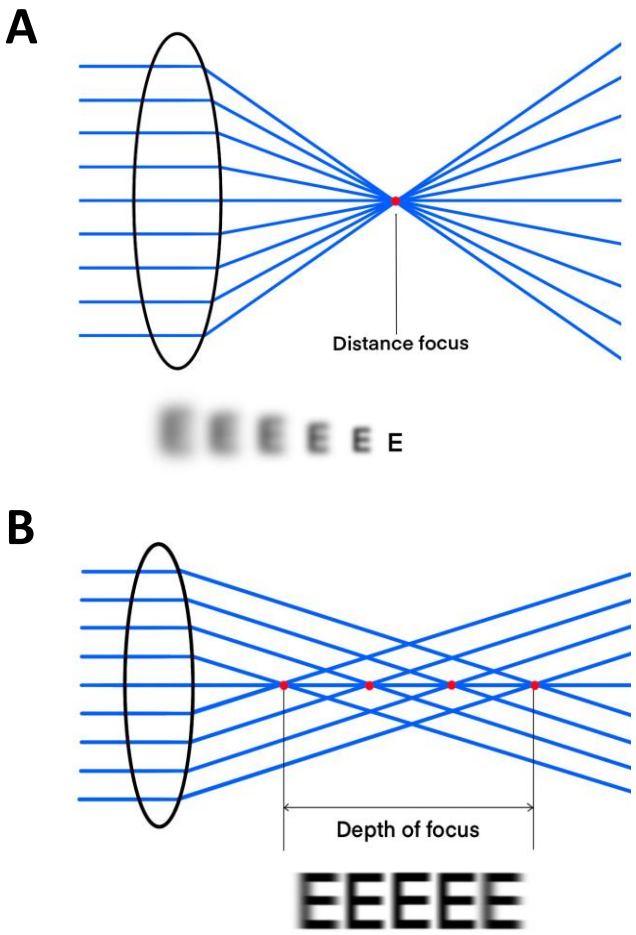


Figure 2. *Ray tracing representation of a single vision CL (A) and a multifocal CL (B). The single vision CL creates a single focal point, for which the optical quality is optimal, but progressively decreases out of this point. In the multifocal CLs, multiple focal points are created, elongating the depth of focus (DOF). The optical quality along this DOF is not optimal, but it is acceptable, providing multifocality.*

This process involves several photic phenomena or dysphotopsias, common reasons for complaints regarding presbyopia-correcting CL wear. As expected, the image quality will be affected and will depend on several factors, either relative to the lens designs or the wearer characteristics. Therefore, visual performance analysis is crucial concerning these CLs.

1.2.2.1.Designs

Through the years, several designs to correct presbyopia have been used, but rotationally symmetric ones are currently the most accepted and generally known, leaving translational designs in second place (23). Originally, diffractive CLs consisting of a Fresnel-type eschelete pattern were the main design, but the significant deterioration of visual performance left them out of the market. Nowadays, the main optical designs can be broadly classified into the following categories (24):

- Concentric or annular CLs

Concentric lenses are formed by a circular central viewing zone for distance, surrounded by one or more concentric rings containing a near add, or vice versa. Concentric designs are considered non-monotonic (the power profile changes abruptly across the optic zone) and periodic in nature (the distinct zones for distance and near are equally separated across the lens). The central diameter varies to improve either distance or near vision (23).

Additionally, some concentric designs, instead of a constant power in each ring, present a smooth parabolic decrease towards the negative direction from the centre to the periphery, which is thought to allow for the effects of the usually positive spherical aberration (SA) of the natural eye, technically improving near vision in the centre and distance vision in the periphery

(25). As expected, optical performance with concentric zonal CLs will vary with pupil size, ocular SA and diffraction, since these factors affect the effective add power (26).

- Aspheric CLs

Aspheric CLs are designed by a smooth and progressive change in the power from the centre to the periphery in one or both surfaces of the lens. Aspheric lenses are considered monotonic in nature, which means they provide a gradual change in power distribution from the centre to the periphery of the optic zone. SAs are induced by this asphericity: positive SA when the power increases as the radius increases (centre-distance designs, CD) and negative SA when the power decreases as the radius increases (centre-near designs, CN) (see Figure 3) (23).

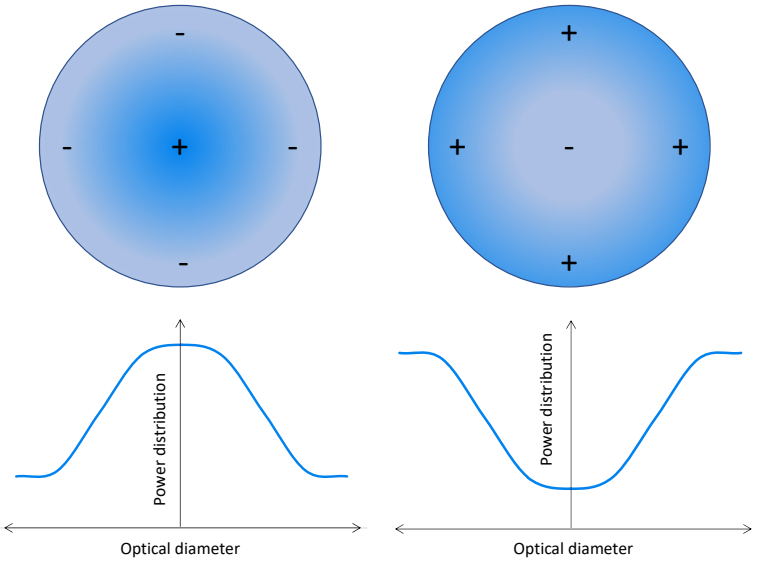


***Figure 3**. Front view (up) and power profile (down) of CN (left) and CD (right) aspheric CL designs.*

Power profiles for low add lenses show this progressive change from the centre to the edge because they are single-aspheric. For bi-aspheric lenses, power profiles show an abrupt change following two different parabolic functions between the central and the outer zone (25). This asphericity across the optic zone and the consequent multiple images focusing on the retina induce an enhanced DOF, which provides acceptable optical quality over an extended range of vergences. However, visual quality is somehow compromised by the increase in SA (23).

- Extended depth of focus (EDOF) CLs

Extended depth of focus is a different relatively novel design of CLs, although most multifocal designs already intend to do this by the introduction of SA terms (e.g., aspheric designs). In contrast to other designs, EDOF lenses present a non-monotonic, non-aspheric, aperiodic, non-diffractive, refractive power distribution, provided by the deliberate manipulation of higher-order SA terms (27). This was suggested by some authors, who proposed that extending the depth of focus of a CL by combining primary and secondary SA with different signs could be a useful strategy for presbyopic lens designs (28).

Theoretically, EDOF lenses are less affected by pupil size variations (i.e., pupil-independent), inherent ocular aberrations and CL decentration (29). Additionally, EDOF designs are thought to overcome the common limitations of conventional designs, such as decreased contrast sensitivity, halos, glare, or ghosting, and showed to provide significantly intermediate and near vision compared to other designs, with no compromise of distance vision (27,30).

1.2.2.2.Visual performance with presbyopia-correcting CLs

Aside from the design complexity, presbyopia-correcting CLs must provide acceptable vision either with or without residual accommodation in the case of younger or older presbyopes, respectively. As previously mentioned, nowadays CL designs rely on the simultaneous-image principle to provide the desired multifocality and allow the wearer to see at all distances. However, simultaneous image, along with the extension of the DOF, are the main factors responsible for the reduced image quality during wear, mostly because of the out-of-focus image and the division of light into two or more foci. This results in visual disturbances and dysphotopsias, such as reduced contrast sensitivity, ghosting, haloes, and glare perception (31,32). However, it should be noted that blur tolerance varies among individuals and that some kind of adaptation to multifocality is expected over time (14).

It is known that visual acuity (VA) is an important measurement to assess vision, but does not represent visual performance on its own. More tests are required to establish a correct analysis of real visual function, like contrast sensitivity, through-focus or defocus curves, or patient's subjective quality of vision, among others (23). Different lighting levels should also be considered. Additionally, it should be noted that these measures will differ from monocular to binocular performance, which depends on the multifocal design or ocular dominance.

Accordingly, several reviews regarding visual performance with presbyopia-correcting CLs have already been published (22–24,33,34). Besides differences in visual performance among studies regarding designs or luminance conditions, showing that each CL perform differently from the others (35), presbyopia-correcting CLs provide satisfactory results.

1.2.2.3. Fitting considerations

Visual performance strongly relies on several factors affecting the final fitting success: pupil dynamics, age and aberrations (the combination of the inherent patient's and the CL aberrations). Knowing this information could potentially increase the percentage of presbyopes successfully fitted with a presbyopia-correcting design.

- Pupil size and age

As previously mentioned, pupil size is a key factor when prescribing presbyopia-correcting CLs. It is known that pupil diameter decreases with age (36), along with other factors such as the accommodative reflex and the usually photopic conditions during near activities (37,38). Luminance has been found to be the main influential factor altering pupil size (36). Additionally, several authors have shown a close relation between pupil size and optical and visual performance with these CLs (39–41).

As mentioned in the description of the different designs, some of them are more susceptible to pupil size than others. Aspheric designs are fully pupil-dependent, since they present a gradual change in power distribution from the centre to the periphery of the optic zone. On the other hand, EDOF designs are theoretically pupil-independent thanks to the manipulation of multiple higher-order SA terms.

Moreover, it has been shown that the pupil-power profile interaction has a decisive effect on the distance correction and the add power, and it should be noted that this interaction varies between patients of the same age and for the same patient under different lightning levels (42). Advanced presbyopes will need higher add powers than early presbyopes, which means a larger power gradient across the lens. Additionally, myopes, emmetropes and early presbyopes usually present larger pupil diameters compared to hyperopes and older presbyopes

(36). In this context, the latter will need a faster progression of power distribution across the optic zone (in the case of aspheric designs) to meet the requirements for an acceptable vision at all distances.

- Aberrations

It is widely known that the inherent ocular aberrations of the eye closely interact with the aberrations of the lens itself (22). Due to changes in the crystalline lens shape with age, SA terms have been shown to move towards negative values. Nevertheless, the inherent SA of the population varies among a wide range of values, even opposite in sign. Therefore, the combination of both inherent and lens aberrations plays an important role regarding on-eye lens performance. In fact, some authors have found that when fitting the exact same design in eyes with the same refractive error but different SA, visual performance significantly differed (41).

Visual performance will depend, in part, on the different levels of SA created by the different presbyopia-correcting designs (43). As mentioned above, most fitted designs (e.g. aspheric and EDOF designs) are based on the introduction of SA into the optical profile to provide the desired multifocality and extended depth of focus. Therefore, depending on the eye's natural aberrations, the patient may experience an increase or a neutralisation of the total SA.

1.2.2.4. Comfort

When a CL is fitted on the eye, it challenges the ocular surface structures. The division of the tear film into a pre- and post-lens tear film causes several changes to take place mostly in the pre-lens layer, such as thinning of the lipid layer, reduction in tear volume and increase in evaporation rate (44). These alterations turn into significant differences in clinical parameters, such as increased bulbar hyperemia and lissamine green staining (45), worsened tear break-up times (46) and an overall less stable pre-lens tear film. These changes induced by CL wear, along with those related to ageing, can interfere with CL fitting success and reduce its tolerance in terms of discomfort, and might eventually lead to CL dropout.

According to the Tear Film and Ocular Surface Society (TFOS), contact lens discomfort (CLD) is "*a condition characterized by episodic or persistent adverse ocular sensations related to lens wear, either with or without visual disturbance, resulting from reduced compatibility between the contact lens and the ocular environment, which can lead to decreased wearing time and discontinuation of contact lens wear*." (44)

Although pre-lens tear film parameters are associated with CLD, it cannot be said that a single one is responsible for CLD on its own, since data suggest that tear film parameters are interconnected (44). Despite recent improvements in lens materials and care solutions over the years, CLD is still considered a major issue in clinical practice. It is estimated that between 12% and 51% of CL wearers stop wearing lenses, with CLD as one of the primary reasons (44).

CLD is influenced by several intrinsic lens factors, such as lens material and design, and lens fitting. Moreover, subject's inherent characteristics and environmental factors, such as ocular environment and external environment should also be taken into consideration when fitting a CL (see Figure 4) (44).

As expected, age is considered a significant factor influencing CL retention rate (47), and some authors have reported a greater decrease in comfort in presbyopes during wear when

compared to non-presbyopes (48). Nevertheless, other authors suggest that presbyopes should not be overlooked for CL fitting, although the process will probably be more challenging compared to a younger population.

Varying some of the factors affecting CLD, such as changing care solutions, wearing schedules, lens material, or the size and shape of lens edges could help in the management of CLD. However, that is not straightforward. Changing some of the parameters of the lens directly affects the others, and few manufacturers offer the option to change only one parameter (44).

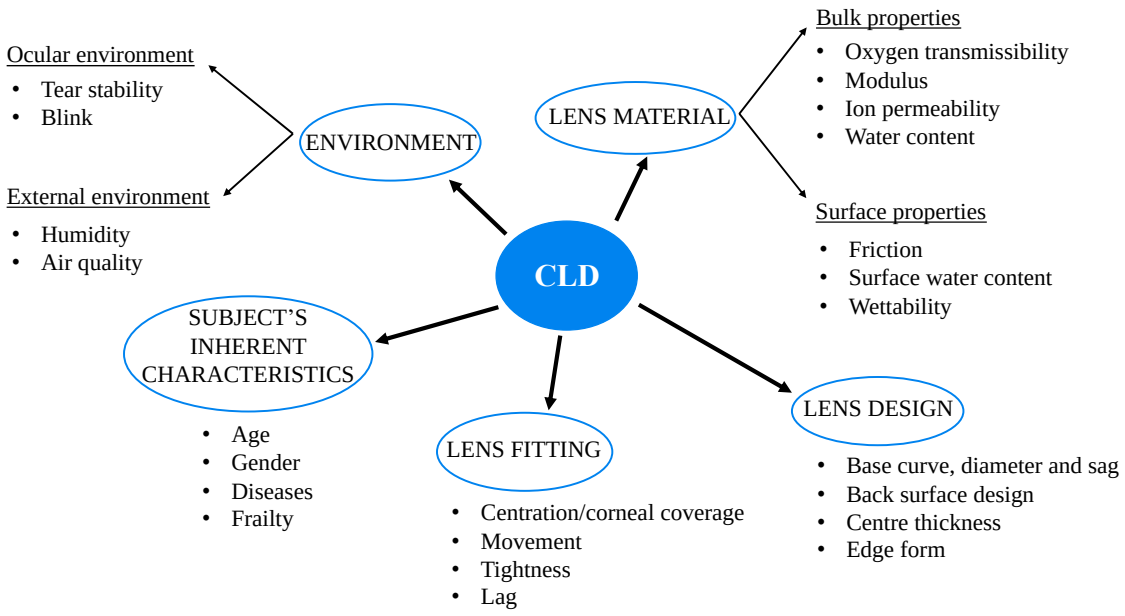


***Figure 4**. Factors influencing contact lens discomfort (CLD).*

Moreover, as a subjective response, comfort can be challenging to measure. Dry eye questionnaires might be useful to understand the character of CLD since dryness is a major contributor (49). The matter appears when ocular symptoms cannot be associated with clinical signs, which is the main issue regarding CLD management and diagnosis (50). In fact, as mentioned above, CLD is primarily characterized by ocular symptoms and sensations related to the patient's experience, not by clinical signs, and its aetiology is still undetermined (44). The question arises whether the symptomatology of CLD might be correlated to some underlying inflammatory process induced by CLs given the potential evidence for frictional wear, which will be discussed in following sections.

As mentioned in this section, CLs are a suitable alternative for correcting presbyopia, although they have some limitations. Even though CL dropout is most commonly attributed to CLD and vision problems, handling issues are reported as one of the main reasons for discontinuation (51–53). In fact, 41% of CL wearers who stopped wearing CLs considered easier handling as a decisive factor to change if they were to try CLs again (53).

Additionally, along with the physiological changes in the crystalline lens causing presbyopia, the ageing process ultimately leads to cataract formation. Decreased and blurred vision, glare, reduced contrast sensitivity and VA, ghosting, monocular double vision and increased myopia are the main complaints (54)

Thus, there is a point when presbyopes are no longer able to manipulate CLs, and CLs, even with a perfect lens-eye interaction and visual fitting, cannot provide the desired vision. At this point, intraocular lens implantation is the main approach, which will be addressed in the following section.

1.2.3. PRESBYOPIA-CORRECTING INTRAOCULAR LENSES

The traditional method to restore vision after cataract surgery was the implantation of a monofocal intraocular lens (IOL). However, monofocal IOLs to correct distance vision required spectacles for any other distance. Bifocal IOLs were developed to create two focal points of clear vision, normally for far and near distances. Yet, the gap between those two distances did not allow good intermediate vision, limiting the patient's range of daily activities.

Nowadays, the presence of multifocal and EDOF IOLs to offer good vision at different distances and spectacle-independence is a major contributor to the improvement in the quality of life and vision of presbyopic patients after cataract surgery. Thanks to the research in the field of cataract surgery and IOL implantation over the past decades, a wide variety of lenses are currently available in the market, making the surgical process more likely to be successful (55). Figure 5 shows a graphic representation of the Modulation Transfer Function (MTF) vergences of good vision for generic monofocal, bifocal, trifocal and EDOF IOLs.

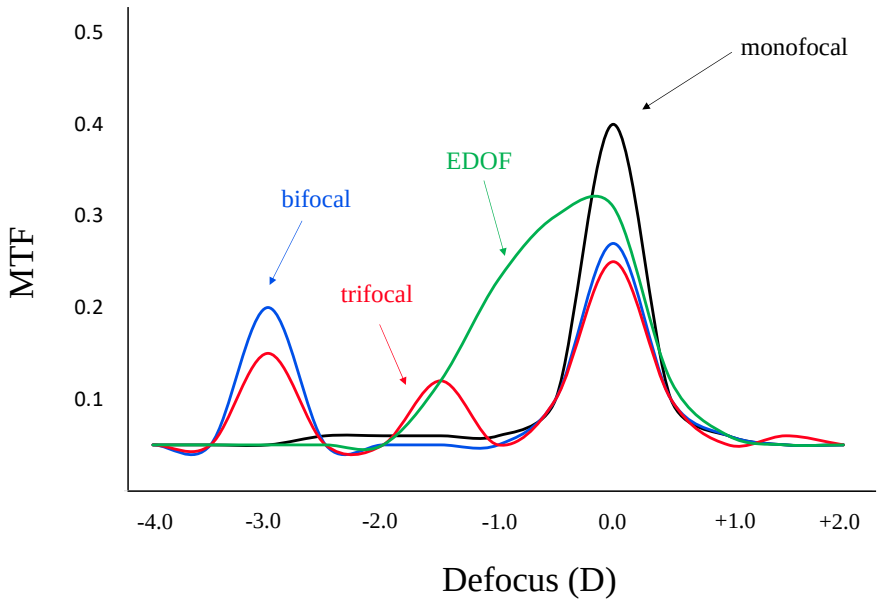


Figure 5. *Simulation of through-focus MTF for monofocal (black), bifocal (blue), trifocal (red) and EDOF (green) IOLs.*

Given the wide variety of IOL designs to correct presbyopia, lens selection is a key factor regarding postoperative success. Patient selection also plays a crucial role in this process. Surgical success will mean identifying the right "psychological" and the "physiological" candidate for each lens, although there is no "perfect" candidate for either (55). Educating the patient preoperatively and postoperatively to identify the limitations of these IOLs will also improve the result.

As in CLs, the multifocality process is given by the simultaneous image principle. A neuroadaptation process is necessary to achieve good visual performance (56), although it may vary among patients (57). Unwanted photic disturbances may be present due to the light division process and the blurred image accompanying the clear image. Postoperative follow-up visits are important to check whether the patient adapts to this mechanism, or if these photic phenomena are persistent.

Different options can be considered as alternatives for presbyopia correction with IOLs: accommodating IOLs or presbyopia-correcting IOLs. Currently, the latter alternative (including both multifocal and EDOF designs) is the most commonly used method among surgeons.

1.2.3.1.Designs

The main optical principles used for correcting presbyopia with IOLs are refraction and diffraction, or a combination of both (hybrid lenses). The selected design will depend on different factors, such as the patient's lifestyle, age, needs, preoperative clinical condition, pupil dynamics, economic status and expectations, and surgeon's preference and experience (56).

- Refractive IOLs

Refractive IOLs are formed by concentric rings with different radii of curvature, focusing light on different focal points for different distances (see Figure 6). The main disadvantages of this design are the pupil-dependence, the low tolerance to decentration, the presence of haloes, and the reduction in brightness and VA due to the destructive interference between phases of incoming light (58). In fact, refractive IOLs are especially contraindicated in professional drivers given the reduced visual performance in dim light conditions.

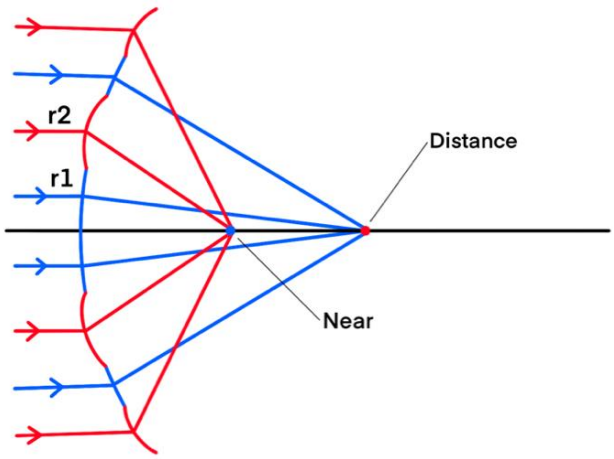


***Figure 6**. Graphic representation of a refractive IOL design. The different radii of curvature form different focal points for different distances. r1 and r2 correspond to distance and near radii, respectively.*

- Diffractive IOLs

Diffractive IOLs are characterised by concentric rings separated by echelettes of about two microns that diffract light into two or more foci. The order 0 of diffraction creates the distance focal point, whereas the order 1 creates the near focal point (see Figure 7). Each echelette contributes to each focal point, making diffractive designs pupil independent, a major advantage of this design. The higher the echelette, the more light for the near foci, and the

wider the echelette, the higher the addition. Diffractive IOLs can be apodised by progressively reducing the height of the diffractive steps from the centre to the periphery of the lens (59). Apodization helps in distance vision when the pupil is dilated (smaller echelettes in the periphery) and in near vision when the pupil is contracted (higher echelettes in the central zone). The main disadvantages of diffractive designs are the visual disturbances at night and the diminished contrast sensitivity due to the diffraction principle.

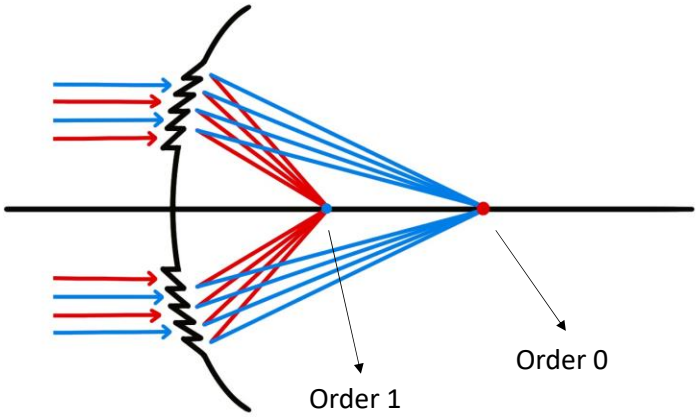


Figure 7*. Graphic representation of a diffractive bifocal IOL design. Each echelette diffracts light into different orders, creating two or more focal points. The order 0 represents the distance focal point, and the order 1 represents the near focal point.*

- Hybrid IOLs

Hybrid IOLs are a combination of both refractive and diffractive designs to reduce their drawbacks and combine their advantages. Hybrid IOLs can also include apodisation, with a progressive decrease in step height across the lens until they reach a refractive zone (58). Some hybrid designs show typical features such as pupil-independency, smooth transitions between zones and aberration correction.

- EDOF IOLs

Both refractive and diffractive designs produce dysphotopsias, resulting in visual disturbances that might affect patient satisfaction. Just like CLs, the "extended depth of focus" technology has also been introduced into IOLs. In contrast to the other multifocal IOLs designs, which aim to create two or three discrete focal points (bifocal and trifocal designs, respectively), EDOF IOLs aim to enhance the range of distances with good VA by creating a single-elongated focal point (60) (see Figure 8).

This extended DOF can be achieved by either narrowing the aperture (pinhole effect) or using high-order aberrations (HOAs) (i.e., SA) (61). Besides knowing that the presence of HOAs is normally associated with a worsening in visual quality, these aberrations also widen the depth of focus, which is the main principle of pure EDOF designs. EDOF technology combined with other ways of creating multifocality (e.g., additional power or diffractive or refractive-hybrid profiles) is not considered pure EDOF IOL but a hybrid design (62).

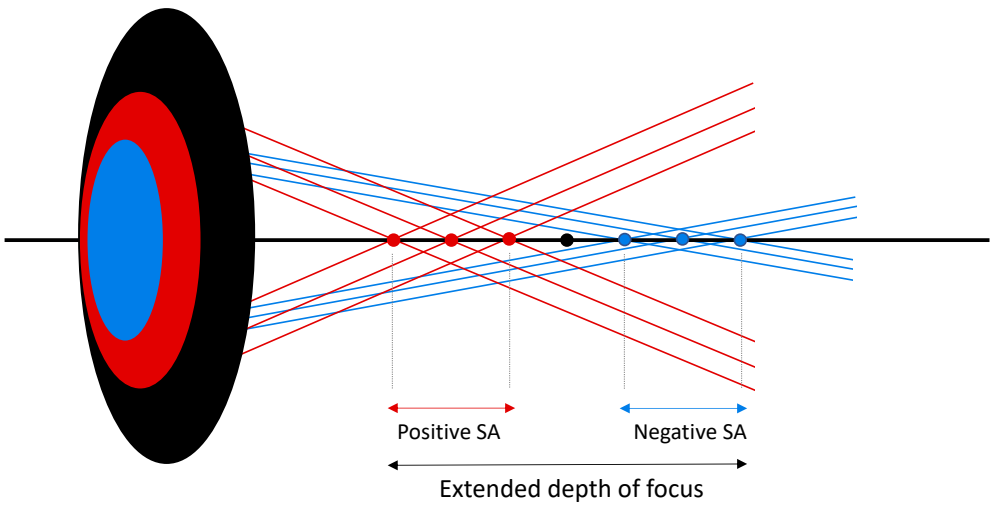


Figure 8. *Extended depth of focus IOL simulation. The deliberate manipulation of SA consists of a central zone with negative SA (blue), an intermediate zone of positive SA (red), and a monofocal zone in the periphery (black).*

EDOF IOLs aim to eliminate the overlapping of distance and near focal points produced by conventional multifocal IOLs, which is thought to eliminate halo perception. Theoretically, EDOF lenses provide improved optical quality along the range of focus compared to other multifocal designs (63).

1.2.3.2.Visual performance with presbyopia-correcting IOLs

Presbyopia-correcting IOLs were developed to compensate for the diminished vision at near and intermediate distances given by monofocal IOLs, with no compromise of distance vision. Accordingly, several reviews and meta-analyses have assessed visual performance, efficacy, and safety with these IOLs. Overall, they have shown to be an effective and satisfactory way of offering spectacle independence (57), which directly improves patient's satisfaction. However, visual performance slightly varies among designs, mainly because of the multiple factors affecting light distribution and different optic designs under the same "name".

One meta-analysis showed that EDOF lenses provided worse near VA, but better contrast sensitivity compared to trifocal IOLs, with no significant difference in halos and spectacle independence (64). Another recent meta-analysis did not reveal significant differences in distance VA between trifocal and hybrid-EDOF IOLs, but a better intermediate uncorrected VA was found in the latter (65). On the contrary, trifocal IOLs performed better at near (corrected and uncorrected visual acuities) but increased halos incidence. Additionally, some authors showed that EDOF lenses seemed to provide satisfactory near and intermediate VA and reduce halos perception compared to other multifocal IOLs (60). Moreover, trifocal

IOLs have shown a clear improvement in intermediate vision compared to bifocal IOLs, although both obtained satisfactory distance vision (66).

Presbyopia-correcting IOL performance can be measured either by subjective methods once the lens is implanted (defocus curves, visual acuities, contrast sensitivity or stereopsis), or by objective methods in an optical bench (through-focus curves, wavefront aberrations, retinal straylight, or MTF). In contrast to CLs, it is not possible to analyse the visual performance of different IOLs and different situations in the same eye. That is why objective optical quality measures are more popular in this field. Additionally, through objective methods, optical quality is not biased by other ocular factors, such as retinal function, neural processing, or the condition of the eye (clarity of the cornea), although they have their own limitations.

As can be observed in the scientific literature, given the wide variety of presbyopia-correcting IOL options, these lenses are currently extensively studied. Several studies have evaluated the visual and optical performance of the different designs in "normal" patients, and so far, they seem to provide satisfactory good, although not perfect, vision at different distances (the correct design in the appropriate patient) (56) and are a safe and effective means of correcting presbyopia (57).

However, there are several additional factors that may cause a decrease in VA and a reduction in contrast sensitivity, apart from the design. Those factors related to the patient (pupil size, previous surgeries, preoperative condition of the cornea), and those related to the surgery itself (residual refractive error or lens decentration, rotation and tilt) can potentially affect visual and optical performance. These specific situations need to be considered and studied to improve knowledge about presbyopia-correcting IOLs and meet the needs and expectations of each individual (67).

1.2.3.3. Implantation considerations and complications

- Pupil size

As in CLs, pupil size and dynamics are considered one of the most influencing factors in choosing a presbyopia-correcting IOL. As previously mentioned, pupil dependence varies with the design. Diffractive IOLs are less pupil-dependent, whereas refractive lenses strongly rely on pupil size. It should be noted that during cataract surgery and lens implantation, pupil size can be affected, and some differences can be found between pre- and post-surgery measurements (56). Additionally, it is known that pupil diameter decreases with age (36). Thus, small pupils will affect near vision, whereas large pupils will result in incremented dysphotopsias (56).

- Preoperative astigmatism

It is known that a spherical IOL implanted in patients with preoperative high corneal astigmatism will compromise VA. This can be solved either by corneal incision techniques during or after surgery, corneal photoablative procedures or toric IOLs. The latter has been shown to be a more predictable and effective procedure (68).

However, the accuracy required in a toric IOL implantation is compounded by the complexity of a multifocal design. A toric presbyopia-correcting IOL must be placed in a determined and specific axis to provide an acceptable vision (69). Any misalignment after IOL implantation can lead to residual refractive error, therefore affecting visual performance and reducing patient's satisfaction. Thus, analysing the effect of IOL rotation after implantation is of great importance.

- Previous corneal refractive surgery

Post-corneal surgery patients represent another specific situation. Laser in situ keratomileusis (LASIK) surgery has increased in prevalence in the past years and is one of the most common refractive surgeries worldwide. More and more people reaching presbyopia who underwent ablation surgery in the past are now in need of cataract surgery and IOL implantation. The particularity of these patients is that they are more likely to have higher expectations regarding spectacle independence, and that the lens calculation becomes more challenging due to the change in the cornea layout (67). Accordingly, analysing these IOL optical performance with different corneal profiles would be of special interest.

- Tilt and decentration

Tilt and decentration of IOLs can result from either a complicated surgery or from an ordinary and routine surgery. These events might potentially have an impact on visual and optical performance of monofocal IOLs, but even more on presbyopia-correcting IOLs, therefore inducing photic phenomena and leading to patient's dissatisfaction (70). Accordingly, a decentred IOL will show changes in light distribution between the different foci (71), and its optical performance will be affected differently depending on the design (72). On the other hand, a tilted IOL will also affect lens performance. As the complexity of the design increases, the sensitivity to decentration and tilt also increases (73). Therefore, special attention should be paid to these matters.

- Residual refractive errors

Although cataract surgery has experienced great progress in the past years, residual refractive error is considered one of the most common causes for complaints and dissatisfaction after IOL implantation (74). These refractive errors can be caused either by inaccuracies in the biometric calculation, IOL power, wrong position of the lens or by extreme cases limiting the calculation formulas (56). In a cohort of over 17,000 cases of cataract surgery, emmetropia was reached in only 55% of eyes planned for that purpose (75). Given that emmetropia is the main goal in cataract surgery, this outcome suggests a further fine-tuning of IOL calculation. Furthermore, the analysis of this residual refractive error after IOL implantation might be useful in considering clinical approaches in eventful surgeries.

1.3. OCULAR SURFACE AND MOLECULAR IMPLICATIONS OF PRESBYOPIA

1.3.1. CHANGES IN THE OCULAR SURFACE WITH AGE

Besides the characteristic loss of functionality of the crystalline lens and the surrounding structures, and the consequent decrease in the accommodative amplitude, the presbyopic ocular environment is also subject to ageing.

During ageing the ocular surface experiences molecular and cellular changes, that besides being the underlying cause of structural changes, favour the inflammatory damage. Altered levels of intrinsic complement proteins and activated products triggered by oxidative stress have been found to take part in the ageing process (76). For instance, harmful oxidative products called advanced glycation end products (AGEs), which are favoured by oxidative stress, have been found in the cornea, lens, and different ocular structures. At the same time, eye inhibitors of metalloproteases (MMPs) are under-expressed. MMPs are extracellular matrix enzymes that control the amount and composition of proteins needed so the extracellular matrix can work correctly (77). MMPs also regulate the tight junctions of the epithelial corneal cells, which are essential for a regular ocular surface barrier (78). Therefore, the low level of MMPs inhibitors triggers the accumulation of molecules into the extracellular matrix, complicating the correct function of the cell. Cellular debris will accumulate in the extracellular matrix, which along with oxidative stress, will favour reactive species formation, damaging the surrounding cells and tissues. It will also translate into an excessive proteolytic activity over the tight junctions, compromising the integrity of the protective barrier of the ocular surface. In this context, MMP-9 has gained popularity as a potentially useful biomarker for ocular surface diseases (78), including dry eye disease (DED) (79), an age-related eye disease.

As a results of these molecular and cellular changes, structural changes are likely to appear in the presbyopic ocular surface. Some of the most common age-related changes taking place in the ocular surface components and system include: tear film dysfunction, Meibomian gland decrease in number, diminution in lacrimal secretion and lacrimal inflammation, decreased nerve density and sensitivity and alterations in ocular surface homeostasis, along with systemic senescence of the immune system (80,81) (see Figure 9).

These physiological alterations result in clinical changes, such as increased bulbar and limbal redness, corneal and conjunctival staining and tear meniscus height, which have shown to positively correlate with age, whereas the Schirmer's test and tear break-up times have shown a negative correlation (82). Additionally, age is also associated with an increased prevalence of DED (83,84).

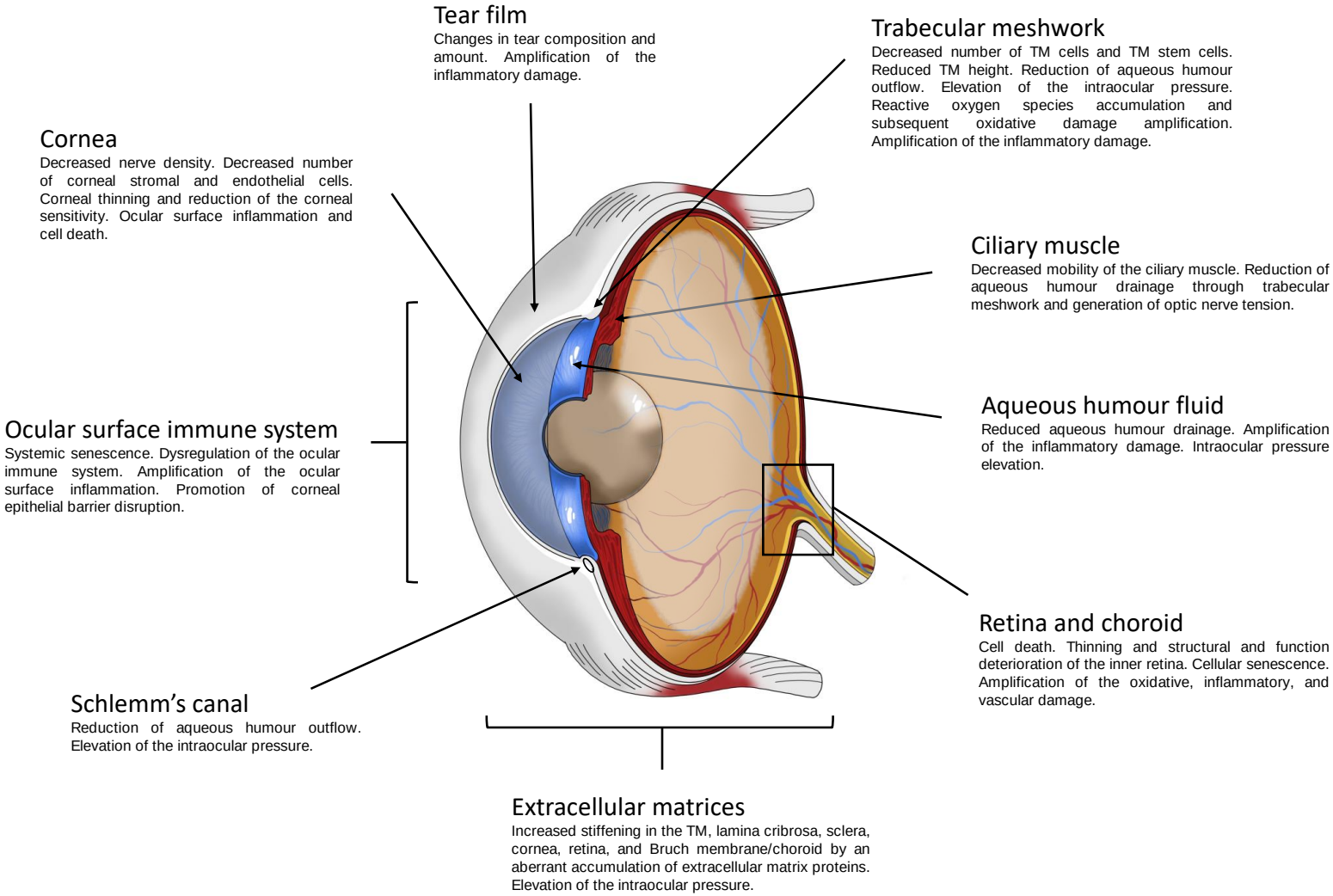


***Figure 9**. Age-related changes taking place in the ocular structures.*

1.3.2. INFLAMMATION AND FRAILTY SYNDROME

It is well-known that the fourth decade of life is a relevant biological period, in which occurs a wave of proteomic changes (85). Cytokines and chemokines are soluble proteins involved in immune-inflammation processes whose expression change in this period (85,86) Therefore, middle-aged individuals overexpress pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumour necrosis factor (TNF)-α, whereas anti-inflammatory cytokines and growth factor, such as IL-10, transforming growth factor (TGF)-β and IL-37 are under-expressed (87). These imbalances are caused partly by the aging-induced alteration of the cells responsible for producing cytokine and inflammatory mediators, immune system cells.

Accordingly, the term "inflamm-ageing" arises as this "reshaping of cytokine expression pattern, with a progressive tendency toward a pro-inflammatory phenotype", and it is believed to be a common process in all ageing, frailty and age-related diseases (87) (see Figure 10). The term "frailty" has been described as "a nonspecific state of vulnerability, which reflects multisystem physiological change". It has been demonstrated that inflammation is a common molecular pathway that underlies most age-related disorders, like frailty syndrome and age-related diseases (88).

Consequently, frailty syndrome is considered a predisposing factor to age-related diseases, including age-related eye diseases (89). In fact, these molecular alterations are the underlying cause of future age-related diseases. Thus, some authors recommend screening inflammatory biomarkers in middle-aged adults before frailty syndrome is fully developed to hopefully prevent this process or, at least, slow down its progression (90)

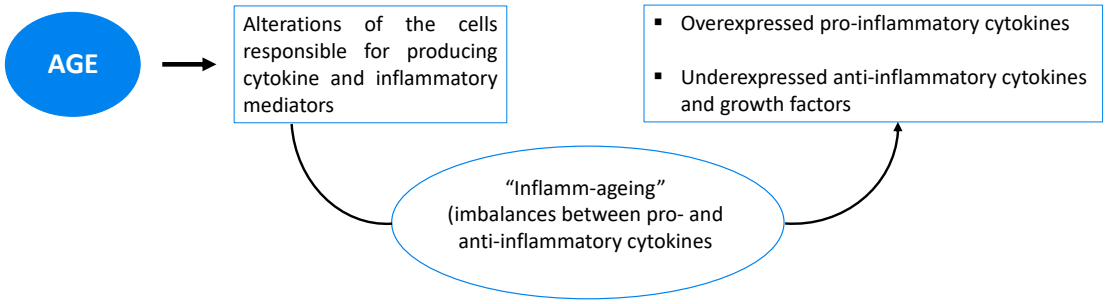


***Figure 10**. Age triggers inflammation through imbalances between pro- and anti-inflammatory cytokines.*

1.3.3. EXTERNAL FACTORS

External factors, such as CL wear, might act as amplifiers of the inflammatory process already present in the presbyopic ocular surface. CLs are known to challenge the ocular structures. To study whether CL wear induce inflammation or not, several molecules of the ocular surface, in particular inflammatory mediators such as cytokines, chemokines and growth factors, have been analysed in tears. Indeed, the results showed that CL wear does affect inflammatory mediators release into the tear film, revealing an underlying inflammatory response (86).

Table 1 summarises some of the studies analysing these inflammatory molecules which experienced significant changes related to soft CL wear. These alterations include significant variations in the expression of inflammatory mediators, such as IL-6 (91,92), IL-8 (91–93), IL-1ß (93–96), TNF-α (96,97), epidermal growth factor (EGF) (97–99), MMP-9 (100), or IL-12p70 (101), among others. This elevated concentration in tears revealed a higher ocular inflammatory response associated with CL wear, which, at the same time, depends on different factors, such as CL material, hours of wear, time of the day, wearing schedule, lens

replacement, environmental conditions, individual's inherent characteristics (age, frailty, etc.). Thus, a wide variety of outcomes can be observed among studies.

Overall, it seems that the physical presence of a CL itself generates a mechanical stimulation associated with the release of inflammatory mediators (98), and that CL wear is intrinsically inflammatory in nature (102).

In this context, some authors have recently applied the term frailty to the ocular morpho-functional unit as a predictive tool for age-related dry eye disease (89). Ocular frailty creates a predisposition for inflammation even before CL fitting. Thus, it is reasonable to think that CLs could be a risk factor for people with frailty since they can trigger and extend an inflammatory process, therefore leading to a more likely age-related disease onset (see Figure 11).

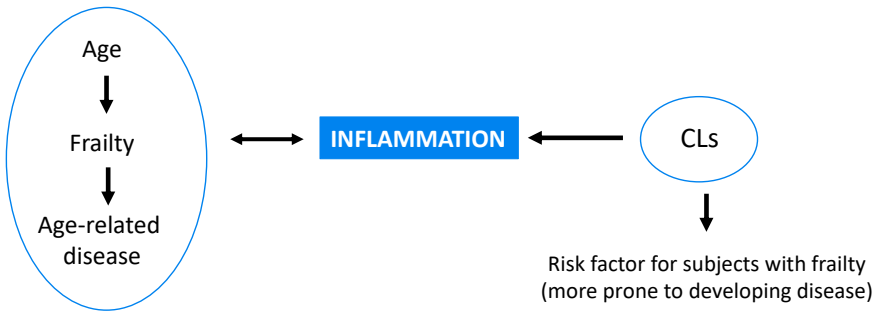


Figure 11. *Diagram of the relation between age, inflammation and CL wear. Inflammation is a common pathway in all age, frailty and age-related diseases. CL have shown to induce the release of inflammatory molecules in the tear film, and are considered a risk factor for subjects suffering from frailty syndrome.*

Additionally, in DED patients, already an inflammatory disease, CL wear would be contraindicated. In fact, researchers have investigated the connection between CLD and inflammation, although there is some controversy about this association, and it is yet unclear.

Some authors found significant outcomes regarding inflammatory molecules in symptomatic CL wearers compared to asymptomatic ones. Some of these findings are a higher concentration in tears of Leukotriene B4 (LTB_4) (103); a correlation between IL-17A and CLDEQ-8 score (104); a higher concentration of TGF- ß1 in CL associated dry eye (105); an overexpression of C-C Motif Chemokine Ligand 2 (CCL2), IL1A, IL1B, IL2 and nerve growth factor (NGF) (106); and significant increases in IL-2, IL-12p70, IL-7, IL-8, IL-13, IL-15, IL-11, EGF receptor, IL-1 receptor antagonist, among others cytokines, in tears of CL-induced dry eye wearers (107).

On the other hand, some authors claim the opposite, that no correlation exists regarding CLD and inflammatory molecule expression. Willcox et al. (94) found no correlation between any cytokine and ocular comfort scores, Saliman et al. (101) showed that none of the parameters measured was associated with comfort during CL wear, except for a weak negative correlation with IL-1ß, Martín-Montañez et al. (97) found no effects of the symptom-based grouping for any molecule analysed, and López de la Rosa et al. (108,109) concluded that the change in the tear concentration of inflammatory molecules did not induce CLD.

Overall, although CL wear has been shown to change the expression of certain inflammatory cytokines, more research is needed regarding whether the release of inflammatory mediators is a contributor to CLD (110,111). The analysis of the balance between pro-inflammatory and anti-inflammatory mediators might be useful to further study this matter (96).

In conclusion, the inflammation characteristic of the ageing process, along with the additional inflammation induced by CL wear could potentially reduce CL tolerance and lead to permanent dropout.

Table 1. Inflammatory molecules in the tear film with significant changes associated with soft contact lens wear.

Authors (year)	Comparison	Molecule analysed	Results (mean ± SD pg/mL)
Chao et al. (2017)(96)	Reusable CLW vs daily disposable CLW	IL-1ß	26 ± 7 vs 16 ± 11
		IL-6	42 ± 14 vs 25±20
		IL-10	83 ± 23 vs 49 ±36
		IL-17A	93 ± 26 vs 54 ± 44
		TNF-α	312 [171 to 468] vs. 189 [6 to 447 (median [range])
Thakur and Willcox (2000)(92)	NCLW vs neophyte CLW vs adapted CLW (at 3, 5 and 8 hr of sleep)	IL-6	Neophyte CLW vs adapted CLW (after 3 hr of sleep): 65 ± 39 vs 175 ± 140 Neophyte CLW vs adapted CLW (after 5 hr of sleep): higher in adapted CLW. Neophyte CLW vs adapted CLW (after 8 hr of sleep): 60 ± 32 vs 218 ± 45 NCLW vs neophyte CLW (after 8 hr of sleep): 147 ± 112 vs 60 ± 32
		LTB_4	Neophyte CLW vs adapted CLW (after 3 hr of sleep): 1372 ± 482 vs 912 ± 174 Neophyte CLW vs adapted CLW (after 8 hr of sleep): 745 ± 70 vs 1148 ± 323 NCLW vs Adapted CLW (after 3 hr of sleep): 1346 ± 346 vs 912 ± 174 NCLW vs neophyte CLW (after 8 hr of sleep): 1005 ± 2015 vs 745 ± 70
		GM-CSF	Neophyte CLW vs adapted CLW (after 8 hr of sleep): 72 ± 27 vs 58 ± 32

			NCLW vs neophyte CLW (after 3 hr of sleep): 45 ± 23 vs 82 ± 19
		IL-8	Neophyte CLW vs adapted CLW (after 3 hr of sleep): 31 ± 19 vs 122 ± 95 Neophyte CLW vs adapted CLW (after 5 hr of sleep): higher in adapted CLW. Neophyte CLW vs adapted CLW (after 8 hr of sleep): 70 ± 32 vs 229 ± 175 NCLW vs Adapted CLW (after 3 hr of sleep): 24 ± 17 vs 122 ± 95 NCLW vs Adapted CLW (after 5 hr of sleep): higher in adapted CLW. NCLW vs neophyte CLW (after 8 hr of sleep): 148 ± 103 vs 70 ± 32
Barba-Gallardo et al. (2018)(93)	NCLW vs neophyte CLW	IL-8	Control vs 1 week: 0.007 ± 0.003 vs 0.07 ± 0.02 Control vs 1 month: 0.007 ± 0.003 vs 0.31 ± 0.05
		IFN-γ	Control vs 1 week 0.16 ± 0.09 vs 2.40 ± 0.37 Control vs 1 month 0.16 ± 0.09 vs 2.55 ± 0.43
		IL-1ß	Control vs 1 day: 0.07 ± 0.04 vs 0.38 ± 0.09
Willcox et al. (2015)(94)	NCLW vs CLW	IL-1ß	Morning NCLW vs CLW group 1: 3.4 ± 0.5 vs 3.2 ± 0.6 (log pg/ml) Evening NCLW vs CLW group 1: 3.3 ± 0.6 vs 3.1 ± 0.9 (log pg/ml) Morning NCLW vs CLW group 2: 3.1 ± 1.4 vs 2.8 ± 1.2 (log pg/ml) Evening NCLW vs CLW group 2: 3.0 ± 1.7 vs 2.4 ± 1.3 (log pg/ml)

		IL-4	Evening NCLW vs CLW group 2: 3.6 ± 1.1 vs 3.0 ± 1.4 (log pg/ml)
		IL-5	Evening NCLW vs CLW group 2: 4.0 ± 1.1 vs 3.3 ± 1.3 (log pg/ml)
		IL-6	Morning + evening combined NCLW vs CLW group 1: 4.3 ± 0.7 vs 4.0 ± 0.8 (log pg/ml)
		IL-8	Morning NCLW vs CLW group 2: 6.4 ± 1.5 vs 6.0 ± 1.1 (log pg/ml)
		IL-13	Evening NCLW vs CLW group 2: 3.9 ± 1.0 vs 3.2 ± 1.2 (log pg/ml)
		TNF-α	Morning + evening combined NCLW vs CLW group 1: 4.8 ± 2.0 vs 4.3 ± 2.2 (log pg/ml)
		VEGF	Morning + evening combined NCLW vs CLW group 2: 4.3 ± 2.3 vs 5.1 ± 1.8 (log pg/ml)
		IL-12p70	Morning NCLW vs CLW group 1: 5.0 ± 0.7 vs 4.9 ± 0.7 (log pg/ml) Evening NCLW vs CLW group 1: 5.2 ± 0.6 vs 4.9 ± 0.9 (log pg/ml) Evening NCLW vs CLW group 2: 5.0 ± 0.7 vs 4.6 ± 0.7 (log pg/ml)
Martín-Montañez et al. (2016)(97)	Baseline vs standard conditions vs adverse conditions for 90 minutes (asymptomatic and symptomatic CLW combined)	EGF	Baseline vs standard conditions: 0.36% change (0.08 to 0.64). Significantly higher concentration in the standard vs adverse conditions
		IL- 1ß	Baseline vs standard conditions: -0.48% change (-0.84 to -0.12). Significantly higher concentration in the adverse vs standard conditions

		IL-2	Baseline vs standard conditions: -0.48% change (-0.87 to -0.09). Significantly higher concentration in the adverse vs standard conditions
		IL-6	Baseline vs adverse conditions: 0.35% change (0.09 to 0.62). Significantly higher concentration in the adverse vs standard conditions
		TNF-α	Significantly higher concentration in the adverse vs standard condition
Poyraz et al. (2012)(91)	Baseline vs 6 months of SiH or conventional hydrogel (CH) CL wear in CL neophytes.	IL-6	SiH group: 7.1 ± 6.4 vs 33.1 ± 15.0 CH group: 10.9 ± 8.2 vs 34.4 ± 17.5
		IL-8	SiH group: 107.4 ± 56.8 vs 412.6 ± 104.1 CH group: 176.4 ± 59.7 vs 456.7 ± 19.2
González-Pérez et al. (2012)(99)	Control vs 6 months of SiH CL wear.	EGF	698.1 ± 271.6 vs 1536.6 ± 492.6 (median ± SD)
Markoulli et al. (2013)(100)	SiH CL neophytes	MMP-9	Extended wear at midday baseline vs 1 month: 6.6 ± 9.9 vs 16.9 ± 19.6 Extended wear on awakening baseline vs 1 day: 2123.3 ± 1762.8 vs 3598.7 ± 3229.1
		LCN2	Extended wear at midday 1 day vs 1 month: 192.6 ± 59.6 vs 351.9 ± 136.9
		TIMP-1	Daily wear on awakening baseline vs 1 day: 256.2 ± 139.0 vs 396.6 ± 221.7
Yüksel Elgin et al. (2016)(95)	Control NCLW vs SiH-CLW	IL- 1ß	Control vs SH: 58.32 ± 3.61 vs 69.28 ± 9.05
		GM-CSF	Control vs SH: 1144.43 ± 49.78 vs 1026.52 ± 53.82

Saliman et al. (2020)(101)	Reusable CH vs Reusable SiH vs DD SiH (1 week of CL wear)	IL-12p70	Reusable CH CL vs DD SiH CL: 8.8 (5.5. to 12) vs 3.7 (0.6 to 6.8) (interocular difference between control and test eyes) Reusable SiH CL vs DD SiH CL: 8.9 (5.7 to 12.1) vs 3.7 (0.6 to 6.8) (interocular difference between control and test eyes)
Kallinikos et al. (2006)(98)	Control vs SiH (12 months of CL wear)	EGF	579.4 ± 436.0 vs 1382.9 ± 680.4

CH: conventional hydrogel; CL: contact lens; CLW: CL wearer; DD: daily disposable; EGF: epidermal growth factor; GM-CSF: granulocyte-macrophage colony-stimulating factor; IFN: interferon; IL: interleukin; LCN2: lipocalin 2; LTB4: Leukotriene B4; MMP: metalloproteinase; NCLW: non-CL wearer; SiH: Silicone hydrogel; TIMP-1: tissue inhibitor of metalloproteinase 1; TNF: tumour necrosis factor; VEGF: vascular endothelial growth factor.

JUSTIFICATION

Presbyopia prevalence is rising due to the population ageing and the increased life expectancy (112). Thus, more presbyopes are seeking alternatives to compensate for the loss of accommodation and satisfy the higher visual demands of the 21st century. Main optical spectacle-free options to correct presbyopia include contact lenses and intraocular lens implantation.

In the case of CLs, it is important to evaluate the repeatability of a device before using it to assess clinical parameters. Repeatability is the degree of how close scores or ratings obtained under similar conditions are, that is, the variability in repeated measurements when all factors are assumed constant (113,114). Thus, to properly assess ocular surface parameters, the repeatability of the device must be analysed.

Moreover, the more active lifestyle of the presbyopic population is demanding new CL designs that satisfy its visual demands. It has been hypothesized that there is no "perfect" CLs for presbyopia providing good comfort with no visual compromise (17). Accordingly, EDOF CLs were designed to provide an extended range of clear vision and reduced dysphotopsias, commonly present in other designs. These lenses are theoretically less susceptible to lens decentration, inherent ocular aberrations and pupil diameter variations (27,29,30). Therefore, it is of interest to evaluate visual performance with EDOF CLs.

On the other hand, although vision and comfort are considered the main factors affecting CL fitting success (18), most scientific literature regarding presbyopia-correcting CLs focuses on visual performance and overlook the impact of CL wear on the ageing ocular surface. It is well-known that age does not only affect near work, but it also has several ocular surface implications (81). The presbyopic ocular surface is already compromised even before

CL fitting. Concerning clinical practice, it is of great importance to analyse the impact of CLs on the presbyopic eye.

Besides CLs, IOLs are another alternative to correct presbyopia. Several factors affect visual and optical performance, including the design, the preoperative condition of the eye and surgical events (56). These specific situations are worth studying since they are relatively common. For instance, in the case of preoperative corneal astigmatism, a toric multifocal IOL will be required. In toric lenses, the position of the axis plays an important role in postoperative vision (69). Thus, analysing the tolerance to rotation will be of interest. Residual refractive errors are also frequent after IOL implantation (75), which will affect the optical quality. Moreover, decentration and tilt are common events during IOL implantation that might affect optical performance, even more in presbyopia-correcting designs (71). Additionally, LASIK surgery rate has increased in the past years, and many presbyopes who underwent a previous corneal surgery in the past are now seeking IOL implantation. Therefore, studying the optical performance of presbyopia-correcting IOLs considering these common factors might be useful regarding clinical practice. Given that it is not possible to analyse different situations of IOLs on the same eye after implantation, objective measures with an optical bench are required to assess the optical performance of these lenses.

Moreover, age is a promoter of a progressive decline of each organ specific functions, including the eye, causing alterations in ocular physiological functions. In particular, inflammation is a common pathway in ageing, frailty and age-related diseases (87). It has been shown that CLs are associated with a higher release of pro-inflammatory mediators, which suggests an underlying inflammation (86). The characteristic inflammation during ageing plus the inflammation associated with CL wear might be crucial factors conditioning CL fitting. For these reasons, investigating the potential inflammatory effect of long-term CL wear in

presbyopes, along with the correlation between the molecular changes and clinical damage, might be of special relevance.

Additionally, these inflammatory biomarkers could mediate ocular frailty because they are especially sensitive to CL wear and are associated with age-related eye diseases. Thus, given that some specific biomarkers modulate neutrophil migration and its processes, it would be interesting to study whether neutrophils regulate age-related eye diseases. Moreover, considering that the vast majority of presbyopes will seek presbyopic correction, this moment could be used for early detection of frailty syndrome, avoiding therefore the onset of age-related eye diseases.

Lastly, it is important to reflect on the impact these prevalent diseases have on the public health system and on the individuals' health, and on the opportunity assessing this specific population might represent.

OBJECTIVES

Main objective:

- To evaluate presbyopia-correcting CLs and IOLs and to assess the molecular implications on the ocular surface during this period of life.

Secondary objectives:

- To assess the intrarater repeatability of the ocular surface measurements of a non-invasive device during CL wear.
- To evaluate the visual performance and symptomatology of two CL designs for presbyopia and their impact on the presbyopic ocular surface.
- To analyse the effect of defocus and rotation on the optical performance of a trifocal toric IOL.
- To study the changes in optical quality induced by tilt and decentration of two presbyopia-correcting IOL designs with and without a corneal myopic ablation.
- To analyse the effect of long-term CL wear on genes linked to inflammation in presbyopes and correlate these levels with CL-related clinical changes.
- To analyse the role of neutrophils and their traps in the pathogenesis of age-related eye diseases.

CHAPTER 2.
PRESBYOPIC OPTICAL CORRECTION

This chapter includes 4 studies regarding the analysis of both contact lenses and intraocular lenses for presbyopia correction.

First, the repeatability of the K5M device measurements associated with CL wear is evaluated. Afterwards, this device is used to analyse the presbyopic ocular surface integrity of two different CL designs, along with the evaluation of dry eye symptoms. Furthermore, visual performance is assessed to determine differences between an EDOF CL and a conventional aspheric design.

On the other hand, several situations encountered during IOL implantation are simulated in an optical bench to study their effect on the optical quality of different designs. Post-surgical residual refractive errors, rotations, tilt, decentrations, and pre-operative corneal myopic ablations are assessed via MTF analysis.

2.1. STUDY I. Repeatability of Non-invasive Keratograph 5M measurements associated with contact lens wear

2.1.1. Introduction

Contact lenses, as external agents, are widely known to induce several ocular surface changes. CL wear physically divides the tear film into a pre- and post-lens tear film, leading to thinning of the lipid layer, reduction in tear volume and increase in evaporation, among others (44). This overall less stable tear film alters several clinical parameters such as tear meniscus height (TMH), tear break-up time, or ocular redness (45,46). Accordingly, clinical devices such as the Oculus Keratograph 5M (K5M) (Oculus; Wetzlar, Germany) are developed to monitor these changes. K5M is a corneal topographer with integrated tools to measure TMH, non-invasive keratograph break-up times (NIKBUTs), and ocular redness (both limbal and bulbar), among other parameters, which are particularly relevant in CL wearers (CLW).

NIKBUT is considered a valuable clinical parameter regarding tear film quality for prescribing CLs (115). Likewise, TMH is known to significantly decrease with CL wear (116), and ocular redness is related to hypoxia during CL wear (117). Thus, to properly assess the ocular surface of CLW, clinical devices need to be repeatable to provide a high-quality service, which would be beneficial for the clinical practice.

However, there is confusion regarding the field of error measurement given the wide variety of terms used, such as interobserver variation, observer variation, interexaminer reliability, intertester reliability, repeatability, and more. Accordingly, repeatability is defined as the “degree of how close scores or ratings obtained under similar conditions are”, that is, the variability in repeated measurements when all factors are assumed constant, including the rater (113,114).

Several studies have evaluated the intrarater repeatability of K5M measurements in healthy eyes (118–120) and in eyes with DED (118,121). However, to the best of our knowledge, there is no evidence of this analysis in CL wearers. So, it is also important to determine the number of measurements required in a single assessment to provide an adequate level of repeatability.

Therefore, the aim of this study was to assess the intrarater repeatability of K5M measurements (TMH, NIKBUTs, and ocular redness) in soft silicone hydrogel CLW over 15 days.

2.1.2. Methodology

- **Study protocol and participants**

A prospective study was carried out with CLW. Inclusion criteria included CL wear for 8 hours a day, every day, and age ranging from 18 to 55. Subjects with active ocular allergy, previous refractive surgery, DED or medication known to affect tear film production were excluded from the study. The study was approved by the Institutional Review Board (Annex I) and complied to the Declaration of Helsinki. After explaining the basis of the study (Annex II), all participants signed an informed consent (Annex III). A detailed anamnesis was performed on the first day.

One week of CL washout was required prior to the beginning. Afterwards, all participants were asked to wear a commercial monthly soft silicone hydrogel CL for 15 days. The clinical examination was performed using the K5M device (Figure 12), an advanced corneal topographer with an infrared light source, a built-in real keratometer and a colour camera.



***Figure 12**. Keratograph 5M (K5M) device.*

Three consecutive measurements of each parameter were performed. TMH and ocular redness were repeated with the shortest time possible between recordings, whereas NIKBUTs measurements were taken with at least 120 sec of difference to let the tear film recover to baseline levels.

Measurements were taken at Day 1 and Day 15. Day 1 measurements were performed at baseline (no lens), after 8 hours of wear (with lens) and 20 minutes after lens removal (no lens). Day 15 measurements were performed after 8 hours of wear (with lens) and 20 minutes after lens removal (no lens). The order of the clinical tests was as follows: TMH, ocular redness and NIKBUTs. Measurements were taken only on the right eye of each participant and by the same examiner. Temperature and humidity of the room were controlled (24 ± 1°C and 39% ±

2%, respectively), and the same CL solution was handed to all participants (Optifree Express Mds; Alcon Laboratories, Inc, Fort Worth, TX).

- **Measurements**

The parameters evaluated were TMH, ocular redness (both bulbar and limbal), first non-invasive keratograph tear break-up time (NIKBUT-first), and the average of all break-up times (NIKBUT-average). NIKBUTs and ocular redness scores were automatically obtained from the software device, whereas TMH was manually calculated. Three consecutive measurements were performed for each parameter.

- Tear meniscus height (TMH)

TMH was measured at each visit. A photo of the lower lid margin was taken with the K5M and TMH was manually calculated with a graphic tool as the perpendicular distance from the lower lid margin to the end of the meniscus (see Figure 13).

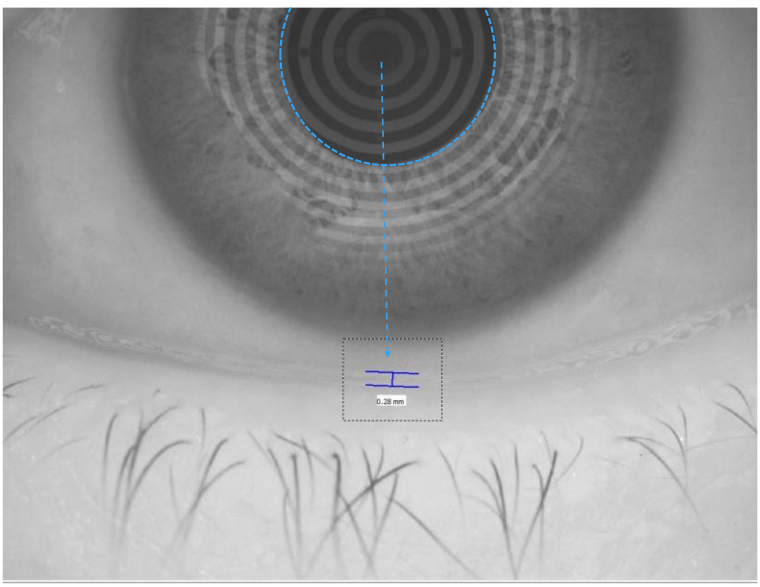


Figure 13. *Tear meniscus height measurement with the K5M device.*

- Ocular redness

Ocular redness was automatically measured with the K5M device, which creates a redness score depending on the ratio between blood vessels and the rest of the surface in line with the JENVIS grading scale (122). The device generates a global redness score and divides the surface into different sections: nasal bulbar redness (NBR), temporal bulbar redness (TBR), nasal limbal redness (NLR) and temporal limbal redness (TLR) (see Figure 14).

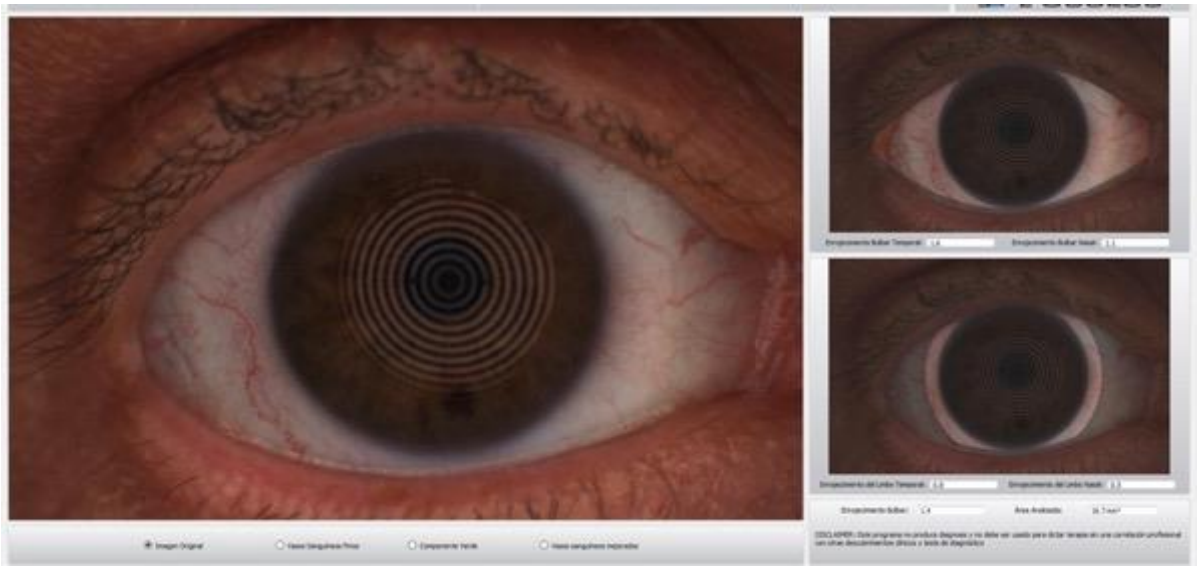


Figure 14. *Ocular redness measured with the K5M device. Temporal (TBR) and nasal bulbar redness (NBR) scores are shown in the upper right corner, and temporal (TLR) and nasal limbal redness (NLR) scores are shown in the lower right corner.*

- Non-invasive keratograph break-up times (NIKBUTs)

First non-invasive keratograph break-up time (NIKBUT-first) and the average of all break-up times (NIKBUT-average) were measured automatically with the K5M device based on perturbation detection of the Placido rings projected on the tear film. Participants were asked to blink twice before maintaining their eyes open for as long as possible after a complete blink.

The device shows a colour-scale graphic representation of the break-up times, with red colours indicating shorter times and green colours indicating longer times (see Figure 15).

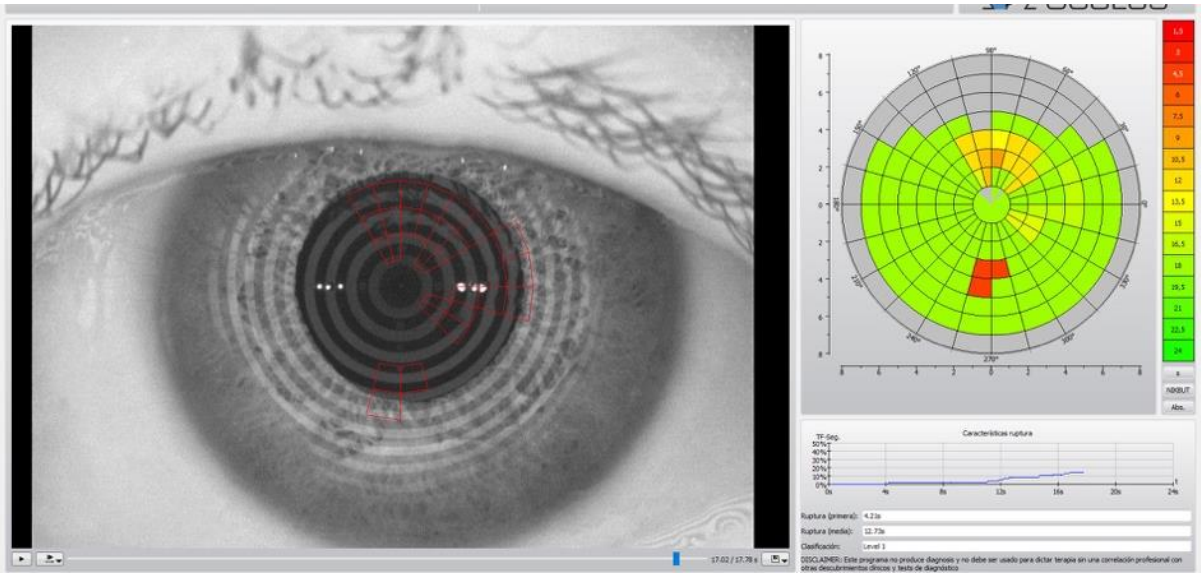


Figure 15. *NIKBUTs measurement with the K5M device (NIKBUT-first and NIKBUT-average values shown in the lower right corner).*

- **Statistical analysis**

The statistical analysis was performed using SPSS statistical package version 23 (SPSS, Inc, Chicago, IL). Normality was checked using the Kolmogorov-Smirnov test. Intrarater repeatability was performed through repeated-measurements analysis of variance and using the statistical parameters shown in Table 2.

According to Koo et al. (123) intraclass correlation coefficients (ICCs) must be reported accompanied by the "model" (1-way random effects or 2-way mixed effects), "type" (single measurement or mean of *k* measurements) and "definition" (consistency or absolute agreement) characteristics. For this work, two different ICCs were calculated: 2-way random

effects, absolute agreement and single measurement [ICC(2,1)]; and 2-way random effects, absolute agreement and multiple measurement [ICC(2,*k*), where *k*=3]. The single-measurement ICC is valid when only one measurement is taken in the clinical practice, whereas the average-measurements ICC is valid when *k* measurements (3 in the case of this study) are taken to calculate the average value. The higher the ICC, the better the repeatability.

***Table 2**. Statistical parameters included in the statistical analysis*

Parameter	**Description**
ICC: Intraclass correlation coefficient	Assessment of the consistency for data sets of repeated measurements within sessions (range 0-1). Calculated based on two forms: single measurement or average of measurements (123) <0.5: poor repeatability 0.5-0.75: moderate repeatability 0.75-0.9: good repeatability >0.9: excellent repeatability
Sw: Within-subject standard deviation (SD)	Estimation of the SD of repeated measurements.
CR: Coefficient of repeatability	Calculated with the Sw. It indicates the interval within which 95% of the differences between measurements are expected to lie (124,125) $1.96 \times \sqrt{2 \times Sw^2}$ or $2.77 \times Sw$
CoV: Within-subject coefficient of variation	Ratio of the Sw to the overall mean. It estimates the amount of variation between repeated measurements taken from the same subject but at different time-points. Smaller values represent higher repeatability.
Intrasubject precision	It indicates the size of the range of error of the repeated measurements for 95% of observations: $1.96 \times Sw$

Sample size was calculated using Granmo v.7.12. sample size and power online calculator based on the difference obtained for TMH measurement (126). Accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, 63 subjects are necessary to recognize as statistically significant a difference greater than or equal to 0.03 units. The standard deviation was assumed to be 0.08. It was anticipated a drop-out rate of 10%.

2.1.3. Results

A total of 64 eyes of 64 patients were enrolled in the study. The mean age of the 48 men and 16 women was 26.3 ± 2.5 years, ranging from 21 to 32 years. Descriptive results for TMH, NIKBUTs and ocular redness scores for the different visits are presented in Table 3.

***Table 3**. Descriptive data of all parameters obtained at baseline, day 1 and day 15 (after 8 hours with CLs and after removing CLs). Data expressed as mean ± standard deviation.*

Parameter	**Baseline**	**Day 1 - 8 hours**	**Day 1 - after removal**	**Day 15 - 8 hours**	**Day 15 - after removal**
TMH (mm)	0.28 ± 0.01	0.25 ± 0.01	0.32 ± 0.02	0.24 ± 0.00	0.26 ± 0.01
NIKBUT- first (sec)	10.56 ± 7.02	10.31 ± 6.75	9.73 ± 6.03	9.67 ± 6.44	9.33 ± 6.40
NIKBUT- average (sec)	12.74 ± 6.54	15.31 ± 5.08	12.39 ± 6.12	15.48 ± 4.90	12.12 ± 5.98
NLR	0.73 ± 0.42	0.75 ± 0.53	0.88 ± 0.52	0.59 ± 0.49	0.80 ± 0.50
TLR	0.46 ± 0.27	0.44 ± 0.30	0.59 ± 0.30	0.38 ± 0.22	0.53 ± 0.32
NBR	1.12 ± 0.44	1.15 ± 0.47	1.20 ± 0.50	0.92 ± 0.39	1.12 ± 0.42
TBR	0.68 ± 0.28	0.74 ± 0.30	0.83 ± 0.27	0.68 ± 0.26	0.77 ± 0.32

TMH: tear meniscus height, NIKBUT: non-invasive keratograph break-up time; NLR: nasal limbal redness; TLR: temporal limbal redness; NBR: nasal bulbar redness; TBR: temporal bulbar redness.

Table 4 shows the ICC ratings (poor, moderate, good or excellent repeatability) for K5M parameters and all situations studied.

Table 4. *ICC ratings for K5M parameters and all situations studied. ICC<0.5: poor repeatability; ICC (0.5-0.75): moderate repeatability; ICC (0.75-0.90): good repeatability; ICC >0.90: excellent repeatability.*

	Baseline	**Day 1- 8 hours**	**Day 1- after removal**	**Day 15- 8 hours**	**Day 15- after removal**
TMH	Excellent	Good-excellent	Good-excellent	Good-excellent	Good-excellent
	Excellent	Excellent	Excellent	Excellent	Excellent
NIKBUT-first	Poor-moderate	Poor-moderate	Poor-moderate	Poor-moderate	Poor-moderate
	Moderate-good	Moderate-good	Moderate-good	Moderate-good	Moderate-good
NIKBUT-average	Moderate-good	Poor-moderate	Moderate-good	Poor-moderate	Moderate-good
	Good-excellent	Poor-moderate	Good-excellent	Moderate-good	Good-excellent
NLR	Good-excellent	Good-excellent	Excellent	Moderate-good	Good-excellent
	Excellent	Excellent	Excellent	Good-excellent	Excellent
TLR	Moderate-good	Good-excellent	Good-excellent	Good	Good-excellent
	Good-excellent	Excellent	Excellent	Excellent	Excellent
NBR	Good-excellent	Good-excellent	Good-excellent	Good-excellent	Good-excellent
	Excellent	Excellent	Excellent	Excellent	Excellent
TBR	Moderate-good	Moderate-good	Moderate-good	Good	Good-excellent
	Good-excellent	Good-excellent	Good-excellent	Excellent	Excellent

Black indicates ICC single measurement results, blue indicates ICC average measurements results. TMH: tear meniscus height, NIKBUT: non-invasive keratograph break-up time; NLR: nasal limbal redness; TLR: temporal limbal redness; NBR: nasal bulbar redness; TBR: temporal bulbar redness.

Table 5 shows the intrarater repeatability analysis under baseline conditions. Considering the ICC-type single measurements, repeatability showed excellent results for TMH [ICC 0.95 (95% CI=0.92–0.96); CR=0.06], good to excellent for NBR [ICC 0.89 (95% CI=0.84–0.93); CR=0.41] and NLR [ICC 0.87 (95% CI=0.81–0.91); CR=0.42], moderate to good for TBR [ICC 0.78 (95% CI=0.69–0.85); CR=0.37], TLR [ICC 0.75 (95% CI=0.65–0.83); CR=0.38] and NIKBUT-average [ICC 0.73 (95% CI=0.61–0.82); CR=9.51], and poor to moderate for NIKBUT-first [ICC 0.57 (95% CI=0.43–0.69); CR=12.77].

On the other hand, considering the ICC-type average measurements (3 consecutive measurements), TMH showed excellent repeatability results, NIKBUT-first moderate to good, NIBUT-average good to excellent, TLR good to excellent, and NLR, NBR and TBR excellent repeatability. Precision levels improved (lower number) for all parameters from ICC single measurement to ICC average measurements (improved with the number of measurements taken). The poorest repeatability levels were found for both NIKBUTs.

Table 5*. Intrarater repeatability of K5M parameters performed under baseline conditions. Data obtained considering 2 intraclass correlation coefficients: single measurement and average measurements.*

	ICC Single Measurement						ICC Average Measurements						
	ICC	**95% CI for ICC**	**Sw**	**CoV (Sw)%**	**Precision**	**CR**	**ICC**	**95% CI for ICC**	**Sw**	**CoV (Sw)%**	**Precision**	**CR**	**Dif CoV (%)**
TMH (mm)	0.95	0.92-0.96	0.02	8.03	0.04	0.06	0.98	0.97-0.99	0.01	4.74	0.03	0.04	3.29
NIKBUT-first (sec)	0.57	0.43-0.96	4.61	43.66	9.03	12.77	0.80	0.69-0.87	3.15	29.87	6.18	8.74	13.78
NIKBUT-average (sec)	0.73	0.61-0.82	3.43	26.94	6.73	9.51	0.89	0.82-0.93	2.19	17.20	4.29	6.07	9.74
NLR	0.87	0.81-0.91	0.15	20.47	0.29	0.42	0.95	0.93-0.97	0.09	12.39	0.18	0.25	8.08
TLR	0.75	0.65-0.83	0.14	30.17	0.27	0.38	0.90	0.85-0.94	0.09	19.07	0.17	0.24	11.10
NBR	0.89	0.84-0.93	0.15	13.01	0.29	0.41	0.96	0.94-0.97	0.09	7.82	0.17	0.24	5.19
TBR	0.78	0.69-0.85	0.13	19.66	0.26	0.37	0.91	0.87-0.94	0.08	12.30	0.16	0.23	7.36

Tables 6 and 7 show the intrarater repeatability parameters analysed in Day 1 and Day 15 considering the 2 types of ICC (single measurement and average measurement). The impact on repeatability during CL wear (after 8 hours of wear) and the impact on repeatability of CL wear (after removing lens) are assessed.

Table 6 shows the results of all repeatability measures at Day 1 after 8 hours of wear before removing the lens (top) and after removal (bottom). During CL wear, the level of repeatability considering ICC-type single measurement can be regarded as good to excellent for TMH [ICC 0.91 [95% CI=0.87–0.94]; CR=0.08], as poor to moderate for NIKBUT-first [ICC 0.40 (95% CI=0.25–0.56); CR=14.44], as poor to moderate for NIKBUT-average [ICC 0.36 (95% CI=0.20–0.52); CR=11.25], as good to excellent for NLR, TLR and NBR, and good for TBR [ICC 0.80 (95% CI=0.71–0.86); CR=0.38]. Considering ICC average measurements, TMH improved to excellent repeatability, both NIKBUTs improved to moderate to good, NLR, TLR, and NBR improved to excellent, and TBR to good to excellent.

After lens removal, TMH repeatability considering ICC single values was classified as good to excellent, NIKBUT-first as poor to moderate, NIKBUT-average as moderate to good, NLR as excellent, TLR and NBR as good to excellent, and TBR as good. Considering ICC average measurements, TMH improved to excellent, NIKBUT-first to moderate to good, NIKBUGT-average to good to excellent, TLR and NBR to excellent, and TBR to good to excellent.

Table 7 shows the results of all repeatability indexes measured at Day 15 after 8 hours of wear before removing the lens (top) and after removal (bottom). During wear, TMH level of repeatability considering ICC-type single measurements was ICC 0.90 (95% CI=0.85–0.94); CR=0.06, which can be regarded as good to excellent. Both NIKBUTs showed poor to

moderate repeatability, NLR repeatability was moderate to good, TLR and TBR were good, and NBR showed good to excellent repeatability. Considering ICC average measurements, TMH improved to excellent, NIKBUTs improved to moderate to good, NLR improved to good to excellent, TLR, NBR and TBR improved to excellent.

After lens removal, the TMH level of repeatability considering ICC single measurement was classified as good to excellent, NIKBUT-first as poor to moderate, NIKBUT-average as good, ocular redness as good to excellent. Considering ICC-type average measurement, TMH improved to excellent, NIKBUT-first to moderate to good, NIKBUT-average to good to excellent, and ocular redness to excellent.

***Table 6**. Intrarater repeatability of K5M parameters performed on Day 1 after 8 hours of wear (during CL wear) and after CL removal. Data obtained considering 2 intraclass correlation coefficients: single measurement and average measurements.*

	ICC Single Measurement						ICC Average Measurements						
	ICC	**95% CI for ICC**	**Sw**	**CoV (Sw)%**	**Precision**	**CR**	**ICC**	**95% CI for ICC**	**Sw**	**CoV (Sw)%**	**Precision**	**CR**	**Dif CoV (%)**
Day 1 - 8 hours of CL wear													
TMH (mm)	0.91	0.87-0.94	0.03	11.31	0.06	0.08	0.97	0.95-0.98	0.02	6.73	0.03	0.05	4.59
NIKBUT-first (sec)	0.40	0.25-0.56	5.21	50.53	10.21	14.44	0.67	0.50-0.79	3.87	37.57	7.59	10.74	12.96
NIKBUT-average (sec)	0.36	0.20-0.52	4.06	26.50	7.95	11.25	0.63	0.43-0.77	3.09	20.17	6.05	8.56	6.33
NLR	0.92	0.88-0.95	0.15	19.81	0.29	0.41	0.97	0.96-0.98	0.09	11.76	0.17	0.25	8.05
TLR	0.85	0.79-0.90	0.11	26.06	0.22	0.32	0.95	0.92-0.97	0.07	15.82	0.14	0.19	10.24
NBR	0.91	0.86-0.94	0.14	12.47	0.28	0.40	0.97	0.95-0.98	0.09	7.43	0.17	0.24	5.03
TBR	0.80	0.71-0.86	0.14	18.60	0.27	0.38	0.92	0.88-0.95	0.09	11.54	0.17	0.24	7.07
Day 1 - After removing CL													
TMH (mm)	0.92	0.87-0.95	0.04	12.92	0.08	0.12	0.97	0.95-0.98	0.02	7.66	0.05	0.07	5.27
NIKBUT-first (sec)	0.46	0.29-0.62	4.42	45.46	8.67	12.25	0.72	0.55-0.83	3.19	32.76	6.24	8.83	12.70
NIKBUT-average (sec)	0.67	0.54-0.79	3.50	28.26	6.86	9.70	0.86	0.78-0.92	2.29	18.46	4.48	6.34	9.80
NLR	0.93	0.90-0.96	0.13	15.23	0.26	0.37	0.98	0.96-0.99	0.08	9.01	0.16	0.22	6.23
TLR	0.92	0.88-0.95	0.08	14.32	0.17	0.23	0.97	0.96-0.98	0.05	8.51	0.10	0.14	5.80
NBR	0.92	0.88-0.95	0.14	11.65	0.27	0.39	0.97	0.96-0.98	0.08	6.90	0.16	0.23	4.75
TBR	0.80	0.70-0.87	0.12	14.67	0.24	0.34	0.92	0.88-0.95	0.08	9.10	0.15	0.21	5.56

***Table 7**. Intrarater repeatability of K5M parameters performed on Day 15 after 8 hours of wear (during CL wear) and after CL removal. Data obtained considering 2 intraclass correlation coefficients: single measurement and average measurements.*

	ICC Single Measurement						ICC Average Measurements						
	ICC	**95% CI for ICC**	**Sw**	**CoV (Sw) %**	**Precision**	**CR**	**ICC**	**95% CI for ICC**	**Sw**	**CoV (Sw) %**	**Precision**	**CR**	**Dif CoV (%)**
Day 15 - 8 hours of CL wear													
TMH (mm)	0.90	0.85-0.94	0.02	9.22	0.04	0.06	0.96	0.95-0.98	0.01	5.51	0.03	0.04	3.71
NIKBUT-first (sec)	0.47	0.31-0.62	4.68	48.41	9.18	12.98	0.73	0.58-0.83	3.36	34.73	6.59	9.31	13.67
NIKBUT-average (sec)	0.44	0.28-0.60	3.66	23.65	7.18	10.15	0.70	0.54-0.82	2.67	17.23	5.23	7.40	6.42
NLR	0.76	0.66-0.84	0.24	40.25	0.46	0.66	0.91	0.85-0.94	0.15	25.32	0.29	0.41	14.93
TLR	0.84	0.76-0.89	0.09	23.07	0.17	0.25	0.94	0.91-0.96	0.05	14.11	0.11	0.15	8.97
NBR	0.93	0.89-0.96	0.10	11.19	0.20	0.29	0.98	0.96-0.98	0.06	6.64	0.12	0.17	4.55
TBR	0.83	0.76-0.89	0.10	15.38	0.20	0.29	0.94	0.90-0.96	0.06	9.42	0.13	0.18	5.96
Day 15 - After removing CL													
TMH (mm)	0.92	0.88-0.95	0.02	8.36	0.04	0.06	0.97	0.96-0.98	0.01	4.97	0.03	0.04	3.39
NIKBUT-first (sec)	0.48	0.32-0.63	4.60	49.33	9.02	12.75	0.74	0.59-0.84	3.28	35.16	6.43	9.09	14.17
NIKBUT-average (sec)	0.66	0.53-0.77	3.47	28.64	6.80	9.62	0.86	0.77-0.91	2.27	18.77	4.46	6.31	9.88
NLR	0.91	0.86-0.94	0.15	18.81	0.3	0.42	0.97	0.95-0.98	0.09	11.19	0.18	0.25	7.63
TLR	0.92	0.88-0.95	0.09	16.90	0.18	0.25	0.97	0.96-0.98	0.05	10.03	0.10	0.15	6.87
NBR	0.90	0.84-0.93	0.14	12.28	0.27	0.38	0.96	0.94-0.98	0.08	7.34	0.16	0.23	4.94
TBR	0.91	0.86-0.94	0.10	12.84	0.19	0.27	0.97	0.95-0.98	0.06	7.66	0.12	0.16	5.18

2.1.4. Discussion

Before using any instrument or device to assess clinical parameters, its repeatability must be analysed. It has been shown that such measurements are often incompletely and inadequately reported in clinical research (113), which highlights the importance of this type of studies to improve critical knowledge about commonly used devices, such as K5M.

Repeatability is the degree of how close scores or ratings obtained under similar conditions are, that is, the variability in repeated measurements when all factors are assumed constant, including the rater (113,114). Herein, the repeatability of different measurements of K5M (TMH, NIKBUTs and ocular redness) were assessed using two ICC forms. In this case, the importance of analysing this device relies on its objectivity and minimally invasive measurements, making it especially relevant regarding CL wear. For this aim, those parameters were measured in CLW wearing a monthly Si-Hy CL over 15 days.

Regarding this matter, other studies have assessed the repeatability of K5M measurements in healthy subjects (118). The authors reported a CR of 0.14 mm for TMH, 5.24 sec for NIKBUT-first and 5.60 sec for NIKBUT-average. The ICC was 0.84 (95% CI for ICC 0.75-0.90), 0.75 (95% CI for ICC 0.62-0.84), and 0.78 (95% CI for ICC 0.66-0.86) for TMH, NIKBUT-first and NIKBUT-average, respectively. Considering our results, TMH measurement would allow a single measurement, maintaining excellent repeatability levels under baseline conditions.

On the other hand, Markoulli et al. (119) reported a CR of 11.37 sec and 13.96 sec for NIKBUT-first and NIKBUT-average, respectively, whereas Fernández et al. (120) found limits of agreement of 13.46 sec and 10.34 sec for NIKBUT-first and NIKBUT-average, respectively. Concerning ocular redness, Wu et al. (121) obtained an ICC value of 0.947 for intraobserver repeatability in 30 eyes, and recommended using K5M device for bulbar redness assessment.

Additionally, some authors have studied the repeatability of K5M parameters in dry eye subjects (118) and obtained better NIKBUT intraexaminer repeatability values and a worse TMH repeatability for dry eyes compared to controls. These outcomes revealed that repeatability can be affected by changes in the ocular surface. Thus, knowing that CLs are an external factor affecting the ocular surface structures, repeatability of K5M measurements was assessed during and after CL wear to study whether CL wear can induce changes in the eye that affect the level of repeatability.

In the case of TMH, repeatability during and after CL wear was excellent when three measurements were taken, which should be considered to obtain the same level as under baseline conditions with a single measurement.

The repeatability during wear for NIKBUT-first and ocular redness remained stable. However, the results obtained for NIKBUT-average worsened during CL wear, from good-excellent at baseline to poor-moderate considering ICC average measurements. This could mean that CL are a potential artifact for NIKBUT-average that could affect repeatability. Additionally, time of wear seemed to not affect NIKBUT-average repeatability during CL wear since the values at Day 15 were not worse than at Day 1.

It should be noted that the repeatability of both NIKBUTs is much lower in all situations compared to the other parameters, but in some cases the variable itself does not allow good repeatability outcomes. The term repeatability is based on the concept of repeated and consecutive measurements. However, for NIKBUT measurement a period of time between tests is needed to let the tear film recover to the baseline situation. Here, the concept of repeatability changes, leading to worse precision values and repeatability coefficients than expected. The question arises whether more measurements (more than three) could have yielded better outcomes, which could be considered a limitation of the study. In fact, a low value of ICC might indicate that more measurements are needed. However, it should also be

taken into account the feasibility of performing more than three measurements of NIKBUT in clinical practice, considering the time required to do so.

Another issue that should be discussed is the precision of the measurement when comparing two values. For instance, if two mean TMH values (0.28 mm and 0.31 mm) show statistical significance, but the precision of the variable is 0.04 mm, these means should not be considered significantly different, because the difference between them is lower than the precision of the measurement.

Moreover, it is important to note that repeatability measures should be assessed with repeatability tests. Other statistical tests, such as pared T test or Pearson correlation coefficient are methods for analysing agreement and correlation, respectively, and are not suitable for repeatability measures. Reliability should also be assessed with specific tests. Accordingly, the intraclass correlation coefficient (ICC) used in this study is a much more suitable index for that aim, although more tests are available, such as Bland Altman analysis (127).

It should be taken into consideration that the outcomes of the present study can only be extrapolated to measurements taken by the same examiner, so no comparison between studies should be performed (different examiners). These results are valid for establishing a control of the same subject.

In conclusion, intrarater repeatability of K5M (variability in repeated measurements performed by the same rater when all factors are assumed constant) was stable for TMH, NIKBUTs and ocular redness when the CL was removed from the eye and three measurements were performed. Nevertheless, during CL wear, intrarater repeatability decreased for NIKBUT-average, independent of the time of wear.

2.2. STUDY II. Visual function, ocular surface integrity and symptomatology of an extended depth of focus and an aspheric contact lens.

2.2.1. Introduction

As presbyopic population increases (112), a rise in CLs to correct presbyopia is expected over the next years. However, regardless of the recent developments in lens options and designs, it has been estimated that the vast majority of presbyopes (approximately 63%) are fitted with a non-presbyopic optical correction (17).

This significant under-prescribing has been hypothesized to be the consequence of the lack of a "perfect" CLs providing both good comfort and satisfactory visual performance (17). As a matter of fact, presbyopes have reported vision and comfort as the main factors influencing CL discontinuation (18). However, most studies analysing CLs in presbyopes focus on visual performance rather than on evaluating the impact CL wear on the ageing ocular surface.

Regarding this matter, it is widely known that the presbyopic ocular surface is compromised due to ageing, even before CL fitting. Some age-related changes in the ocular surface are increased bulbar and limbal hyperemia, corneal and conjunctival staining, tear meniscus height, and shorter tear break-up times (128). These changes are exacerbated when a CL is fitted on the eye, causing an overall less stable tear film. Therefore, it is of great relevance to study the ocular surface of a presbyopic eye when a CL is fitted and the symptomatology of the CL wearer, since comfort is a major contributor to fitting success (18).

Regarding vision, it is well-known that the simultaneous-image mechanism, despite providing the desired multifocality, is the main responsible for unwanted photic phenomena,

such as haloes, glare, decreased contrast sensitivity and overall diminished quality of vision (31,32).

To overcome these limitations, new designs are being developed with the aim of extend the depth of focus by deliberately manipulating high order SA terms and reduce the dysphotopsias associated with other designs (27). Theoretically, extended-depth-of-focus (EDOF) lenses have shown to be less affected by pupil size variations, inherent ocular aberrations and lens decentration (29), and to provide significantly better intermediate and near vision without compromising distance vision (27,30).

Thus, the aim of this study was to assess visual performance, ocular surface integrity and symptomatology of an EDOF CL designs compared to a conventional aspheric CL over a 15-days follow-up in presbyopic eyes.

2.2.2. Methodology

- **Study protocol and participants**

A prospective, crossover, randomised, single-masked clinical trial was carried out at the Complutense University of Madrid. All participants enrolled in the study signed the informed consent (Annex III) prior to study procedures. The study conformed to the principles of the Declaration of Helsinki and obtained ethical approval from the Ethics Committee of San Carlos University Hospital (Madrid, Spain) (Annex I). Inclusion criteria included age between 45 and 65 years, monocular best-corrected distance VA of 20/20 or better in both eyes, near addition power $\geq$ 0.75 D, astigmatism $\leq$ 0.75 D, no history of ocular allergy, and ocular or systemic disorders known to affect the ocular surface or CL wear.

The study protocol is summarised in Figure 16. A complete examination of the participant was performed at Day 0. A clinical sheet was registered including data from the anamnesis. Monocular and binocular distance subjective refraction and addition power were measured with a standardised ETDRS chart.

Ocular dominance was determined using the +1.50 D blur test. A +1.50 D lens was placed before one eye for a short period and changed to the other eye while the participant was looking at a high-contrast distance letter. The dominant eye was determined as the eye for which the VA was reduced greatest while the lens was before it (129). Corneal topography was registered to obtain keratometry readings, anterior corneal eccentricity and horizontal visible iris diameter for the subsequent CL order.

Clinical assessments were taken at baseline (Day 1) and after 15 days of CL wear (Day 15). Visual performance measurements (defocus curves, contrast sensitivity curves, and haloes and glare perception) were evaluated at Day 15 with each CL during wear. Ocular surface integrity and symptomatology were analysed at baseline and Day 15 with both CLs (after lens removal). All participants randomly wore both CLs and all metrics were performed with both designs, with at least 1 week of washout between lenses. Randomisation was performed by an online software (www.randomization.com).

- **Measurements**

The clinical measurements were divided into three different sections: visual performance, ocular surface assessment and symptomatology. Visual performance section included defocus curves, depth-of-focus (DOF) range, contrast sensitivity function, and halo and glare subjective perception. Ocular surface integrity section included TMH, NIKBUT,

NIKBUT-avg, ocular redness, and corneal and conjunctival staining. Symptomatology was assessed by the OSDI questionnaire.

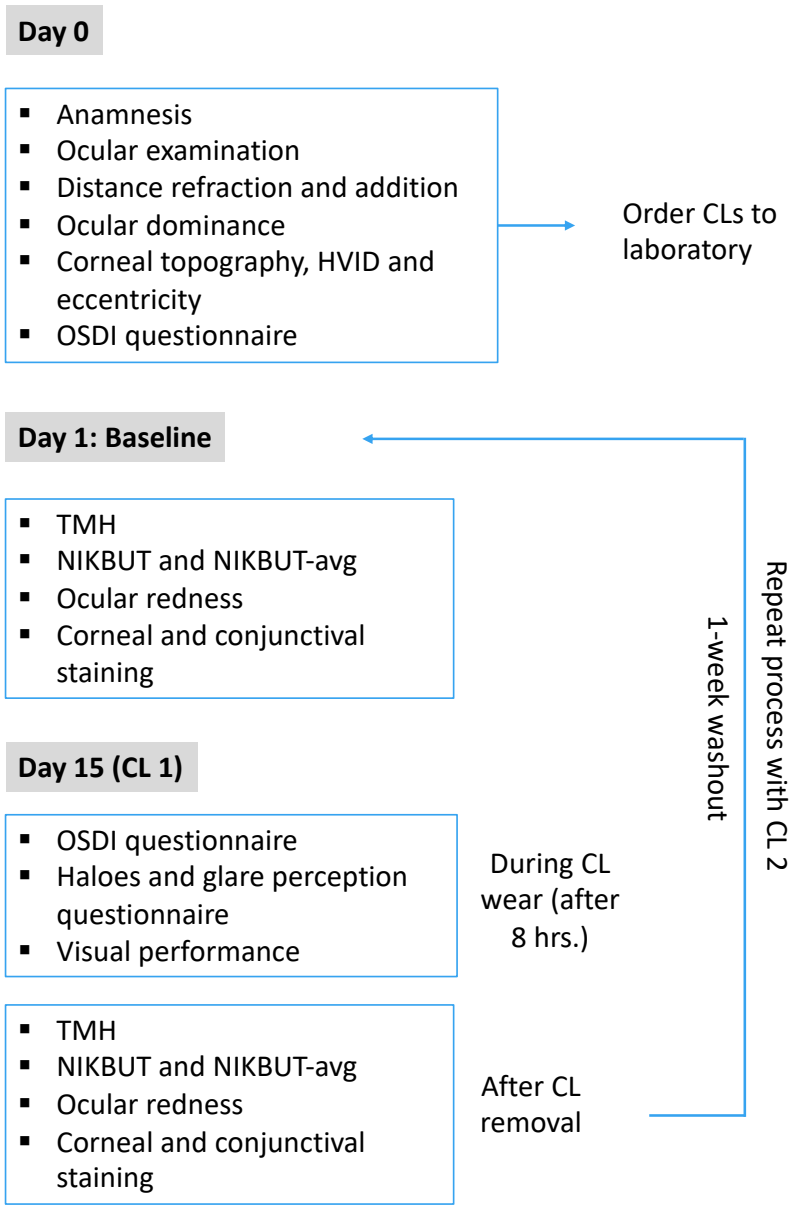


Figure 16*. Study protocol. Measurements performed in each visit. HVID: horizontal visible iris diameter; TMH: tear meniscus height; NIKBUT: non-invasive keratograph break-up time; NIKBUT-avg: average of all break-up times; OSDI: ocular surface disease index questionnaire.*

- Visual performance

- Defocus curves

Binocular defocus curves were measured under photopic conditions (85 cd/m^2) with a distance VA retroilluminated standardised ETDRS chart at Day 15 with each CL. VA was recorded from +1.00 D to -3.50 D in 0.50 D steps with randomised letter sequences. Measurements were registered in logarithmic scale (logMAR).

- Depth-of-focus (DOF) range

DOF range was calculated through defocus curves as the range of vergences obtaining VAs above 0.1 logMAR (0.8 decimal).

- Contrast sensitivity

Binocular contrast sensitivity (CS) curves were measured with the CVS-100 test (VectorVision, Greenville, Ohio) under three different lighting conditions: photopic (85 cd/m^2), mesopic (3 cd/m^2) and mesopic with the effect of glare. Glare was induced by a light source located in both vertical sides of the device. Measurements were taken for 3, 6, 12 and 18 cycles per degree (cpd) and registered in logarithmic units.

- Halo and glare perception

Subjective halo and glare perception were evaluated through a non-standardised questionnaire, which was handled to all participants at Day 15 of each CL (see Figure 17). Frequency (0-3 scale), intensity (1-3 scale) and bothersome (0-3 scale) of the two light disturbances were recorded following the indications of the Table 8. Intensity score ranged from 1 to 3 (no 0 option) because if frequency scored 0, there could not be a 0 intensity.

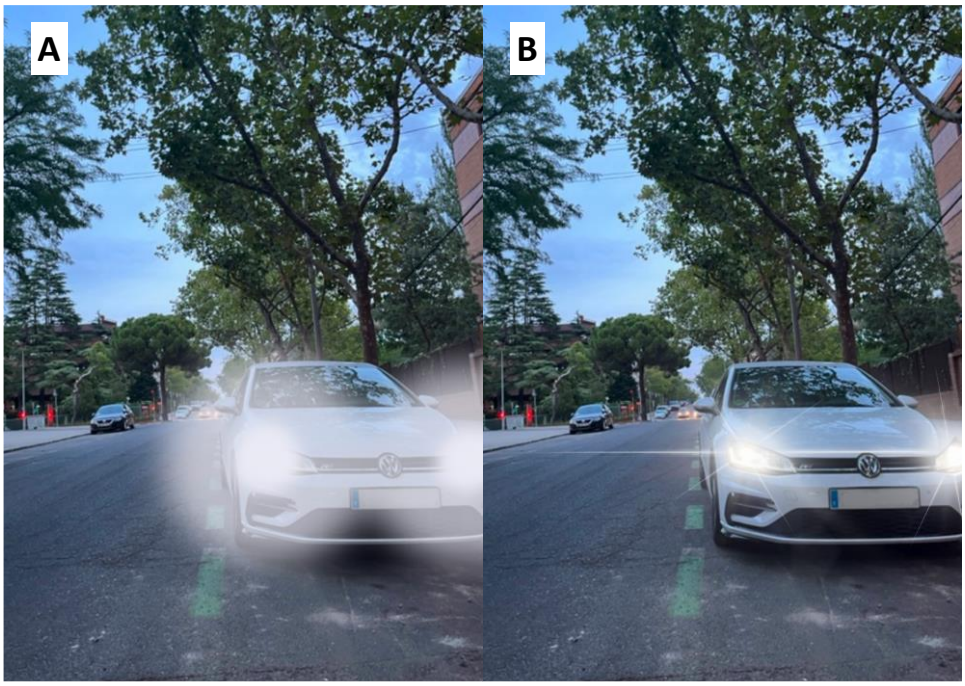


Figure 17. *Halo (A) and glare (B) simulation.*

Table 8*. Halo and glare perception questionnaire score description.*

Frequency (0-3)	**Intensity (1-3)**	**Bothersome (0-3)**
0: Never		0: Not at all
1: Occasionally	1: Mild	1: A little
2: Quite often	2: Moderate	2: Quite
3: Very often	3: Severe	3: Very

The final score was calculated through the following formula on a scale of 0-9:

$$\text{Final score} = Frequency \times \frac{Intensity \times Bothersome}{2}$$

- Ocular surface integrity

- Tear meniscus height (TMH), ocular redness and non-invasive keratograph break-up times (NIKBUT-first and NIKBUT-average), were performed with the K5M device as previously described in Study I (Chapter 2, Study I, Methodology).

- Corneal and conjunctival staining

Corneal and conjunctival staining were graded 2 minutes after the instillation of sodium fluorescein strips (FluoStrip, Contacare Ophthalmics and Diagnostics, Gujarat, India), cobalt blue light and yellow filter according to the Efron scale (130), ranging from 0 (no staining) to 4 (severe staining) (see Figure 18).

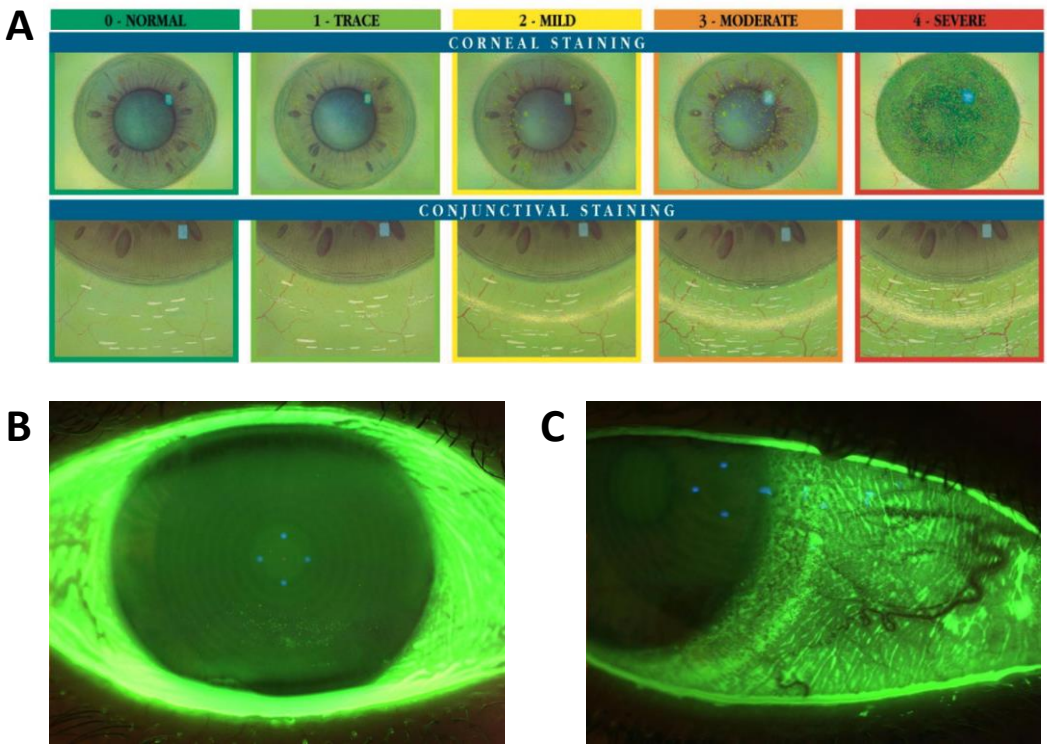


***Figure 18**. Efron scale for corneal and conjunctival staining **(A)**, example of corneal staining **(B)** and conjunctival nasal staining with sodium fluorescein, cobalt blue light and yellow filter **(C)**.*

- Symptomatology

Dry eye symptoms were evaluated with the 12-item Ocular Surface Disease Index (OSDI) questionnaire (131). The questionnaire was distributed to the participants before the rest of measurements to minimise the effect of each clinical test on the subjective symptomatology. The final score was calculated on the basis of the following formula in a scale of 0 to 100. Higher scores indicate an increased severity of symptoms and represent greater disability.

$$OSDI\ score = \frac{\text{Sum of scores for all questions answered x 100}}{\text{Total number of questions answered x 4}}$$

- **Contact lenses**

Two contact lens designs were analysed in this study: a novel EDOF prototype and the commercially available Biofinity Multifocal (MF). Both lenses followed the simultaneous-image principle and were fitted according to the manufacturer's fitting guide. Table 9 summarises contact lens parameters.

EDOF CL has a non-monotonic, non-aspheric, aperiodic, non-diffractive, refractive power profile provided by the deliberate manipulation of high-order aberration terms (27). Its power profile is characterised by different non-equally separated distance and near power zones. Following the manufacturer's fitting guide, three addition powers were used: low (≤ 1.25 D), medium (1.50 - 1.75 D) and high (≥ 2.00 D). EDOF lens parameters (base curve and diameter) were calculated through the manufacturer's automatic calculator according to keratometry readings, horizontal visible iris diameter and eccentricity measurements recorded at Day 0 with the K5M device.

Table 9. *Contact lens specifications.*

	EDOF	**BIOFINITY MF**
Company	Mark'ennovy	Cooper Vision
Material	Filcon V3	Comfilcon A
Water content (%)	75	48
Base curve (mm)	7.10 - 9.80 (step 0.30)	8.60
Diameter (mm)	13.50 - 15.50 (step 0.50)	14.00
Sphere (D)	+18.00 to -18.00 (step 0.25)	+6.00 to -6.00 (step 0.25) -6.50 to -10.00 (step 0.50)
Add power (D)	+0.75, +1.50, +2.25	+1.00 to +2.50 (step 0.50)
Dk	60	128
Oxygen transmissibility (Dk/t)	75 (ct -3.00 0.08)	142 (ct 0.09)
Modulus (MPa)	0.29	0.75

Dk: oxygen permeability; ct: central thickness (mm)

On the contrary, Biofinity MF shows an aspheric, monotonic an aperiodic power profile with a progressive increase in power from the centre to the periphery of the lens (CD design), or vice versa (CN design). Following the manufacturer's fitting guide, for additions > +1.50 D, the dominant eye was fitted with a CD design and the non-dominant eye with a CN design, whereas both eyes were fitted with a CD design for additions ≤ +1.50 D.

For disinfection, cleaning and stage of the CLs, Biotrue® Multi-Purpose solution was provided to all participants.

- **Statistical analysis**

The statistical analysis was carried out using the IBM SPSS package version 25.0 (Inc, Chicago, IL). Sample size was calculated based on the difference obtained for NIKBUT-first measurement between baseline condition and after 8 hours of silicone CL wear in presbyopes (48). Accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, 33 subjects were

necessary to recognize as statistically significant a difference greater than or equal to 1.96 sec. The standard deviation was assumed to be 3.98 sec. Sample size was calculated using Granmo v.7.12. sample size and power online calculator.

After assessing normality of each variable with the Kolmogorov-Smirnov test, a two tailed paired t-test was performed for parametric variables to compared between paired measurements (defocus curves and CS function) and the Wilcoxon test was used for non-parametric variables. Data is expressed as mean ± standard deviation. Statistical significance was set at 95% ($p<0.05$).

2.2.3. Results

A total of 30 presbyopes (20 women and 10 men) were enrolled in the study. 20 participants were classified as high add, 8 as medium add and 2 as low add. Table 10 shows the patient demographic data. Among all participants, 60% were CLW, whereas 40% were non-CLW. None of them were multifocal CLW.

Table 10. *Patient demographics. Data expressed as mean ± standard deviation (range).*

Age (years)	53.7 ± 5.3 (45, 64)
Myopic refraction (D)	-2.18 ± 1.83 (-0.25, -5.50)
Hyperopic refraction (D)	+1.84 ± 1.14 (+0.25, +3.75)
Near spectacle addition power (D)	+1.83 ± 0.40 (+1.00, +2.50)

- **Visual performance**

Figure 19 shows defocus curves at Day 15 and DOF range representation. Defocus curves were similar between CLs, with no significant differences for any vergence between designs (p>0.05 all). The DOF range (range of vergences for which the VA did not decrease below 0.1 logMAR) did not differ between CLs, with values of 3.32 ± 0.61 D and 3.21 ± 0.46 D for EDOF and Biofinity MF, respectively (p=0.521).

Contrast sensitivity function revealed statistically, but not clinically significant changes in low spatial frequencies (3 cpd) under mesopic conditions between CLs (p=0.008), with no changes for the rest of the conditions (p>0.05 all, see Figure 20).

Halo ang glare perception at Day 15 were similar between CLs. Halo results were 1.36 ± 1.88 and 1.30 ± 1.42 units for EDOF and Biofinity MF (p=0.687), respectively, and glare results were 1.05 ± 1.29 and 1.20 ± 1.48 units for EDOF and Biofinity MF (p=0.550), respectively.

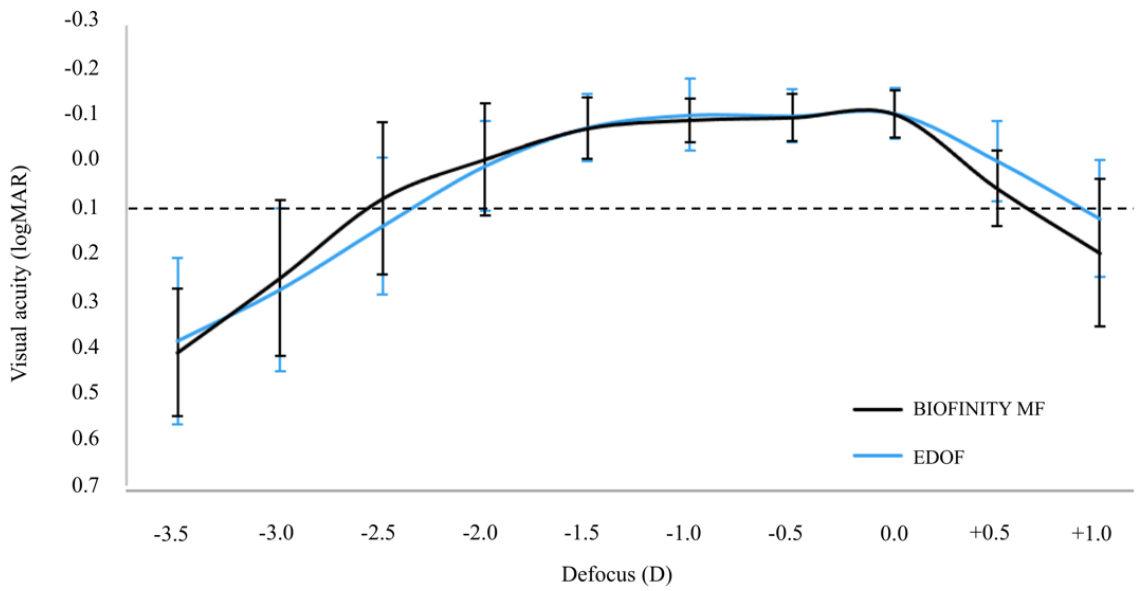


Figure 19. *Defocus curves at Day 15 for both multifocal CL designs, represented as binocular distance visual acuity as a function of the lens defocus (D). Data expressed as mean ± standard deviation. The horizontal dash line is set at 0.1 logMAR (0.8 decimal) and indicates the limit for the DOF range calculation.*

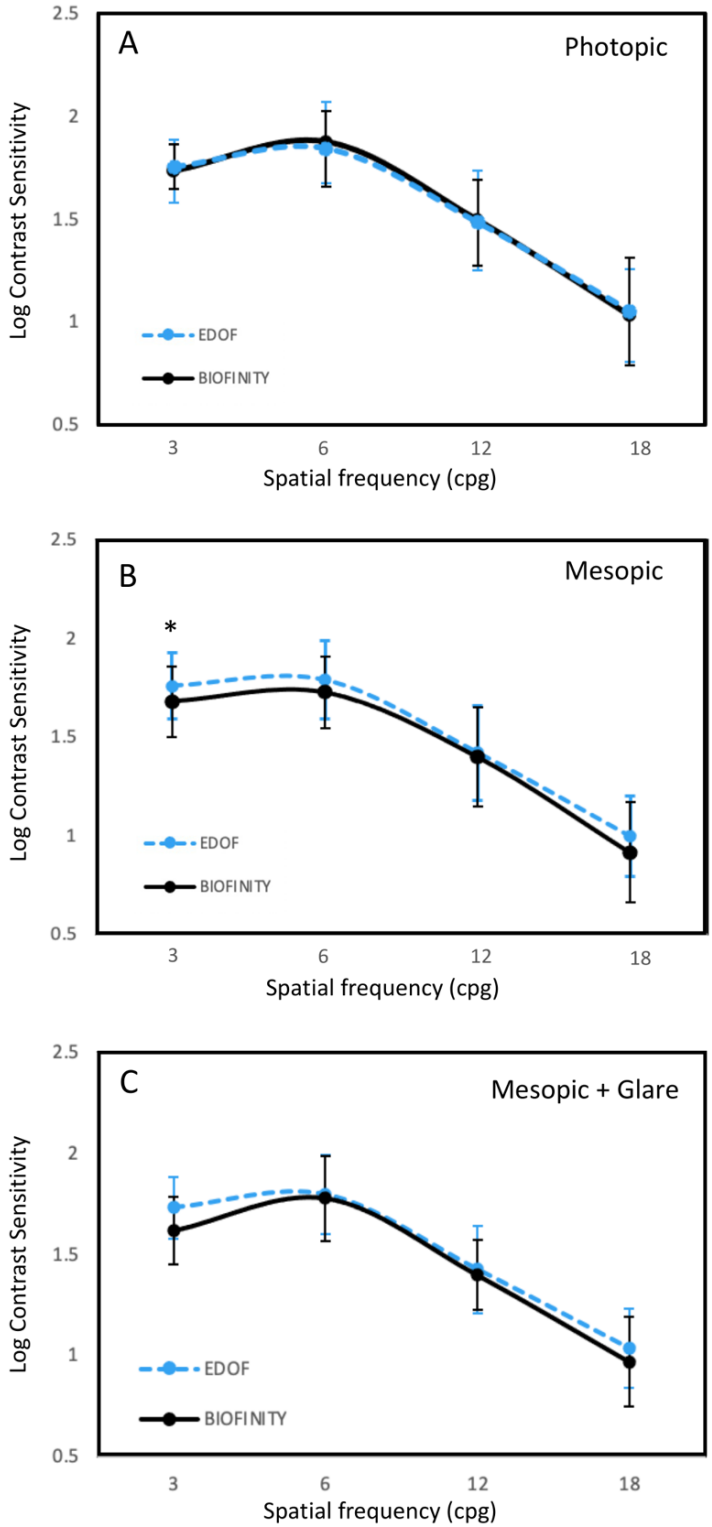


Figure 20. *Contrast sensitivity function at Day 15 under photopic* ***(A)****, mesopic* ***(B)*** *and mesopic and glare* ***(C)*** *conditions. Data expressed in logarithmic units as mean standard ± standard deviation. * indicates $p<0.05$.*

- **Ocular surface integrity**

Table 11 summarises TMH and NIKBUTs results measured with the K5M for both visits and CLs. TMH, NIKBUT-first and NIKBUT-average showed no changes between visits for both CLs (baseline vs. Day 15) ($p>0.05$ all). Redness scores remained constant throughout the study, showing no significant changes between visits for either CL and area analysed (see Figure 21).

Regarding staining outcomes, the percentage of change from baseline to Day 15 was evaluated. EDOF results were as follows: 76.7% of participants showed no change in conjunctival staining score, and the remaining 23.3% increased by 1 point; 86.7% of participants showed no change in corneal staining and the remaining 13.3% increased by 1 point. Biofinity MF results were: 73.3% of participants showed no change in conjunctival staining score, and the remaining 26.6% increased by 1 point; 83.3% of participants showed no change in corneal staining and the remaining 16.6% increased by 1 point.

Table 11. *Ocular surface parameters measured with the K5M at baseline and Day 15. Data expressed as mean ± standard deviation.*

	Baseline	**Day 15**	**P-value**
TMH (mm)			
EDOF	0.29 ± 0.11	0.28 ± 0.13	0.509
Biofinity MF	0.28 ± 0.10	0.27 ± 0.09	0.855
NIKBUT-first (sec)			
EDOF	9.31 ± 7.17	7.83 ± 5.75	0.648
Biofinity MF	8.32 ± 6.15	8.02 ± 5.87	0.776
NIKBUT-average (sec)			
EDOF	12.36 ± 6.43	10.16 ± 5.86	0.191
Biofinity MF	11.34 ± 5.99	11.07 ± 6.26	0.650

TMH: tear meniscus height; NIKBUT: non-invasive keratograph break-up time; NIKBUT-avg: average of all break-up times.

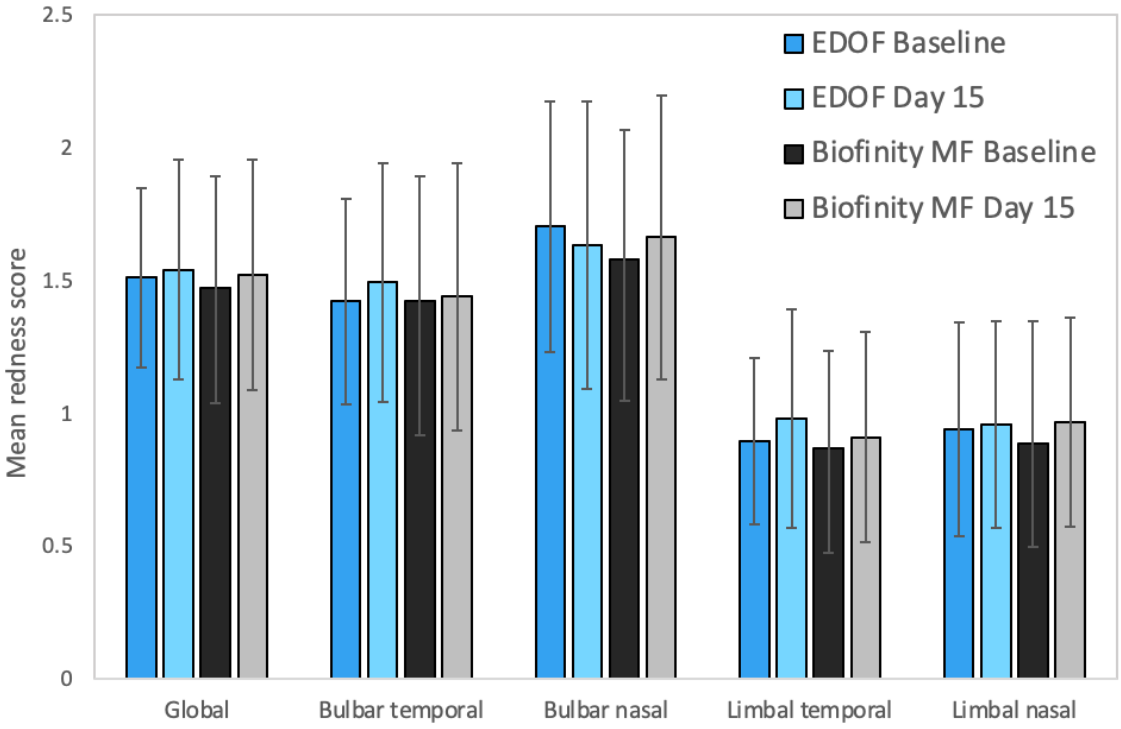


Figure 21. *Ocular redness according to JENVIS grading scale for both CLs at baseline and Day 15. Data expressed as mean ± standard deviation.*

- **Symptomatology**

Both CLs induced dry eye symptoms after wear, but showed no significant difference between baseline and Day 15 for EDOF (p=0.073) and Biofinity MF (p=0.094), and between CLs (p=0.110) (see Figure 22).

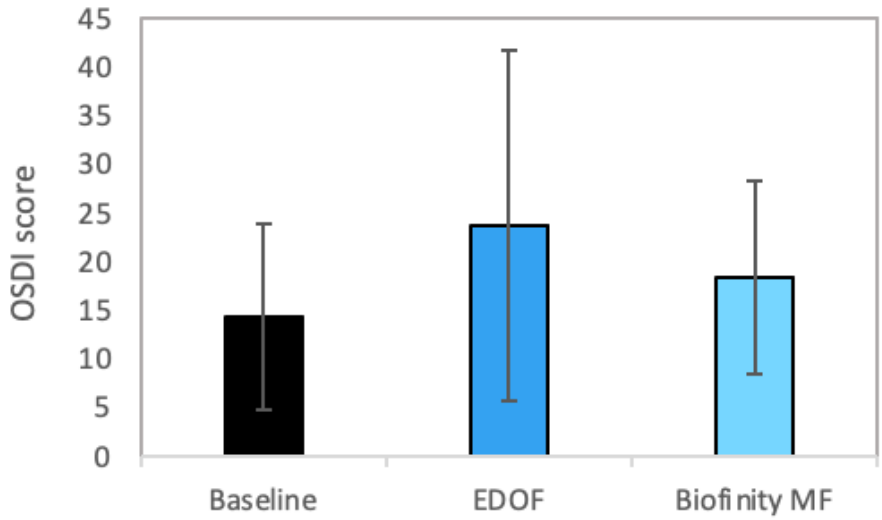


Figure 22. *OSDI score results at baseline and at Day 15 for both CL designs. Data expressed as mean ± standard deviation.*

2.2.4. Discussion

Presbyopia correction with CLs is a major contributor to presbyopes' quality of life, providing spectacle independence and good vision at different distances. However, they are often associated with unpleasant photic phenomena, which diminish visual quality compared to monofocal CLs (31,32). Accordingly, new designs are being developed. EDOF CL has been introduced into the market to compensate for these visual disturbances by creating a single-elongated focal point. Therefore, this study aimed to analyse this novel EDOF design in terms of visual performance and compare it to one of the most fitted CL designs on the current market.

Visual performance outcomes showed no differences between CLs in defocus curves, DOF range, CS curves and photic phenomena perception. The results obtained in this study support that both CL designs provide acceptable visual performance.

These findings are in accordance with a randomised crossover trial conducted by Sivardeen et al. (35). When comparing four different multifocal designs (one of them Biofinity MF), no differences were found at 0.0 D vergence (distance VA), which is consistent with the results obtained in this study.

Regarding defocus curves, Sivardeen et al. (35) found that the different types of lenses worked differently from one another. Accordingly, Figure 19 shows that Biofinity MF and EDOF CLs differed in vergences far from 0.0 D vergence and close to the limits of the DOF range, with Biofinity MF having more tolerance for negative vergences and EDOF for positive vergences. The slightly better performance of Biofinity MF at near distances could be due to the CN design fitted in the non-dominant eye, which would likely provide a larger area of add power in the centre of the lens (25), and to the higher add power available for Biofinity MF. However, despite different performances, no significant changes were found between CLs. As a matter of fact, DOF range values were comparable between designs, provided either by the deliberate manipulation of high order aberration in the case of EDOF, or by the asphericity in the case of Biofinity MF.

To establish a more thorough analysis of visual performance, CS was measured under photopic and mesopic light conditions, and under the effect of glare, which simulates daily life situations, such as night driving. It has been shown that multifocality induces a deterioration of CL visual performance, including CS function (132,133). Additionally, CS is even more affected when a source of light induces glare. García-Lázaro et al. (132) analysed four different multifocal designs (including Biofinity MF) and found no differences for lens type in CS

curves, except for the results for 12 cpd. However, CS was measured with a different device, which might influence the results.

Subjective halo and glare perception were also evaluated, which are known to negatively affect visual performance (134). Similar results were obtained between lenses, which is in agreement with the study conducted by Sha et al., where no differences were found for lack of haloes between lens types (EDOF vs. an aspheric CN design, 1-Day Acuvue Moist Multifocal) (135). A limitation of this study is that the questionnaire was not validated, so other questionnaires could have obtained different results.

Overall visual function outcomes showed that both CLs provide similar performance after 15 days of wear. Additionally, it should be noted that visual performance with these CLs depends on the adaptation time (14), and that longer follow-ups might have probably yielded different results. The benefit of wearing EDOF design would be its pupil-independency, although it was not measured in this study, which should also be considered as a potential limitation.

On the other hand, comfort is still one of the main issues regarding CL wearing satisfaction. The presbyopic ocular surface experiences changes related to ageing, which can be amplified by CL wear. Additionally, vision and comfort have been found to be inter-related in CL wear (136), which underlines the relevance of not ignoring ocular surface evaluation when fitting a CL. Thus, the impact of both CLs on the ocular surface and symptomatology was analysed.

As expected from silicone hydrogel CLs and the same wearing modality, neither of the lenses had an impact on the ocular surface, showing similar results when comparing the baseline situation to Day 15 measurements. TMH, NIKBUTs (NIKBUT-first and NIKBUT-average) and ocular redness scores did not vary with CL wear. The percentage of change after

CL wear regarding staining scores, both corneal and conjunctival, was low and similar between designs. These outcomes are in accordance with a study assessing the ocular surface of presbyopes after wearing multifocal CLs for 4 weeks, where no significant differences in corneal staining and limbal hyperemia were found (35). Another study with presbyopes showed no differences in bulbar redness and NIKBUT-average when comparing baseline measurement with 8 hours of CL wear, although TMH and NIKBUT-first significantly decreased after wear (48).

Once objective ocular surface parameters were analysed, showing that CL wear did not affect the ocular physiology, subjective symptomatology was also measured. To assess symptoms of dryness, the OSDI questionnaire was evaluated at baseline and on Day 15 of each CL. The results revealed no differences between designs on Day 15 and between baseline and Day 15 of each lens, although a not significant increase was observed after wearing the lenses, which is expected due to the mechanical and molecular effect of CL wear in the presbyopic ocular surface. This outcome agrees with the scientific literature, where OSDI scores did not significantly differ between and within groups when comparing three CL designs at baseline, 1 month and 6 months of wear (137).

Although the results of OSDI scores and ocular surface physiology in this study were not significant, they show that CL wear would likely increase dry eye symptoms. Moreover, previous investigations have shown that presbyopes experience a more uncomfortable wearing time compared to non-presbyopes (48), which highlights the importance of properly addressing the presbyopic ocular surface as a more susceptible environment to discomfort. Additionally, multifocal CLs showed to be thicker than monofocal CLs (48), which might exacerbate discomfort symptoms. This brings to light the relevance of material selection and the need for new CL materials or coatings for the ageing ocular surface.

2.3. STUDY III. Effect of defocus combined with rotation on the optical performance of trifocal toric IOLs.

2.3.1. Introduction

Preoperative astigmatism is a frequent situation surgeons encounter in the surgical practice given the high prevalence of pre-existing astigmatism in patients undergoing cataract surgery (138). Preoperative astigmatism is usually corrected with toric IOLs to achieve a proper postoperative uncorrected VA (69). Due to the extremely precise alignment required in toric IOLs, astigmatic correction is considered a challenge. It is known that small variations in the alignment of the lens are associated with decreased visual quality after surgery due to new induced astigmatism (139). Despite providing high rates of stability and good clinical results (69,140), it is not surprising to find some degree of rotation after implantation.

Moreover, when a multifocal toric IOL is implanted, the centration becomes even more relevant regarding visual results (141). It has been observed that rotations over 5 degrees in trifocal toric IOLs lead to a reduction in visual quality, and that this reduction increases with the amount of preoperative cylinder (142).

On the other hand, despite advances in IOL implantation surgery, residual refractive errors are still one of the main causes of unsatisfactory visual rehabilitation (74). In fact, in a cohort of over 17,000 eyes, only 55% of the sample planned for emmetropia reached that aim (75). This emphasizes the importance of considering both astigmatism and residual refractive errors, as key factors for visual success after IOL implantation. Therefore, the aim of this study was to objectively analyse the effect of residual refractive errors combined with determined amounts of rotation on the optical quality of trifocal toric IOLs.

2.3.2. Methodology

- **IOL analysed**

The IOL evaluated was the trifocal hydrophilic FineVision Toric IOL (PhysIOL; Liège, Belgium), with an apodised diffractive trifocal design. It is made of 26% hydrophilic acrylic material (hydroxyethylmethylmethacrylate-comethylmethacrylate copolymer) and presents a refractive index of 1.46 (142). The lens profile presents a combination of two bifocal apodized diffractive patterns for far-near and far-intermediate vision to provide trifocality (142,143), and shows a biconvex-aspheric design with an asphericity of -0.11 µm for a 6-mm pupil. It presents a double C-loop with 5° angulation. It has a total diameter of 11.40 mm and an optic zone diameter of 6.00 mm. The power ranges from +6.0 to +35.0 in 0.50D steps with +1.75 and +3.50 D intermediate and near addition powers, respectively. The sphere of the IOL analysed was +20.0D, with 1.50 and 3.0D cylindric powers as low and moderate-high astigmatism. The lens integrates both ultraviolet and blue-light blocker.

- **Measurements**

To objectively analyse the optical quality, an optical bench was used for the measurements, since it is not feasible to control all variables and conditions in *in vivo* measurements. The PMTF optical bench (LAMBDA-X, Belgium) was used to assess the image quality of the lenses at various frequencies, focal planes, and pupil apertures. The device has the software version 1.13.6 and complies with International Standard Organization 11979-2 and 11979-9 requirements. For each measurement, the device was calibrated to ensure optical

precision. Given that the IOL studied had toricity, the optical bench included specific horizontal and vertical targets for the alignment of the lens.

- Modulation transfer function (MTF)

MTF curves are considered a standard method for the analysis of IOLs optical quality (144–147) since they evaluate the image degradation produced by the optical system (145). MTF measurements are usually extrapolated to visual performance parameters (148). Given that pupil size has a direct impact on light distribution in multifocal designs (149), very small pupils were not considered. Thus a 4.5-mm pupil size was selected for the MTF assessment.

According to previous investigations, the MTF value was considered as the "average modulation value", defined as the average of all frequencies from 0.0 to 100.0 cycles/mm. This number describes the area under the MTF curve from 0.00 to 120.0 cycles/mm (low to high spatial frequencies) (142,143,150–152). Higher MTF values are associated with greater optical quality.

MTF curves were calculated based on three variables:

⇒ Three different positions to evaluate the effect of centration: centred, 5° rotated and 10° rotated.

⇒ Five different positive foci to evaluate the effect of myopic residual defocus: 0.00, +0.25, +0.50, +0.75 and +1.00 D.

⇒ Two different cylindric powers to evaluate the effect of astigmatism: 1.5 D (low) and 3.0 D (moderate-high) cylindric power.

The degradation of the image was evaluated for only one target, given that the principal meridians of the lens and the targets are always perpendicular.

Previous studies have shown that the VA varies 0.18 decimal units every 10 units of average modulation variation (148). Additionally, in previous studies the current IOL showed VA results slightly higher than 20/20 (153). Therefore, the limit of tolerance was set at 10 MTF units (approximately two lines of VA).

2.3.3. Results

MTF outcomes for the trifocal IOL analysed are represented in Figures 23 and 24 and summarised in Table 12.

Figure 23 represents the MTF curves at different object vergences from 0.0 D to +1.0 D for the low cylinder (1.5 D) and for the three different positions: centred (A), 5° rotated (B) and 10° rotated (C). Likewise, Figure 24 represents the MTF curves for the moderate-high cylinder (3.00 D) and for the centred (A), 5° rotated (B) and 10° rotated (C) positions. The MTF curve for the centred position and for a 0.00 D vergence (black line) was established as the reference curve given that it is expected to achieve the best optical performance.

Moreover, Table 12 summarises the average MTF values for the previously mentioned situations between 0.0 and 120.0 cycles/mm. Likewise, the MTF value for the centred position and at a 0.00 D vergence was set as the reference value (indicated by an asterisk).

For both cylindric powers, the MTF average decreases as both rotation and defocus increase. A defocus of 0.25 D for the centred position induced a decrease in MTF of approximately 9 units compared to the reference value, and a similar amount of decrease was observed for the 5° rotated and no defocus situation, showing a certain tolerance to low

amounts of both rotation and defocus. When higher amounts are induced, the MTF decreases over the limit of 10 units (which equate to 0.18 decimal units of VA loss).

***Table 12**. Average MTF values at different focal points and positions (centred, 5° rotated and 10° rotated).*

Vergence (D)	1.5D cylinder			3.0D cylinder		
	Centred	**5° rotation**	**10° rotation**	**Centred**	**5° rotation**	**10° rotation**
0.00	38.58*	28.89	27.02	37.74*	27.07	20.32
0.25	29.25	26.19	21.35	28.96	25.23	19.32
0.50	21.33	18.64	15.18	19.56	18.77	16.17
0.75	15.25	15.15	14.25	15.36	15.38	14.66
1.00	14.72	14.35	14.10	15.32	15.26	14.13

* indicates the reference value.

2.3.4. Discussion

The current study analysed the optical quality of a trifocal toric IOL with two cylindric powers when different amounts of rotation and residual refractive errors are induced. Given the high rate of post-surgical refractive errors and the great impact of rotations on the quality of vision of toric IOLs, the results obtained herein are of utmost importance. It should be noted that these outcomes cannot be directly extrapolated to visual performance in the clinical practice, but they can serve as a reference guide for surgeons who encounter these situations in the surgical practice (154).

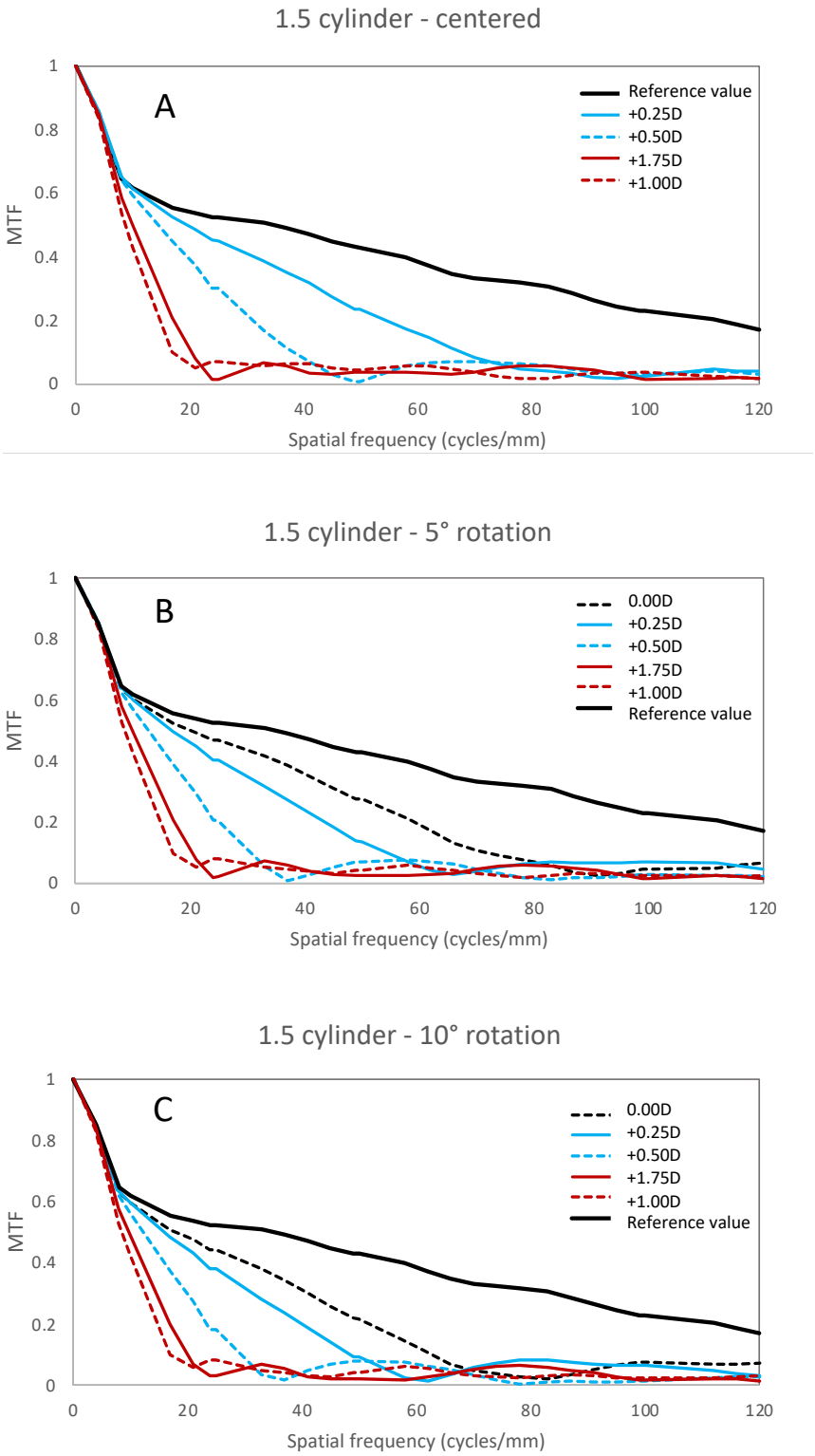


Figure 23. *MTF curves for the low cylinder (1.5D) toric IOL at different vergences (0.00 to +1.00) and at different positions: centred* ***(A)****, 5° rotated* ***(B)*** *and 10° rotated* ***(C)****.*

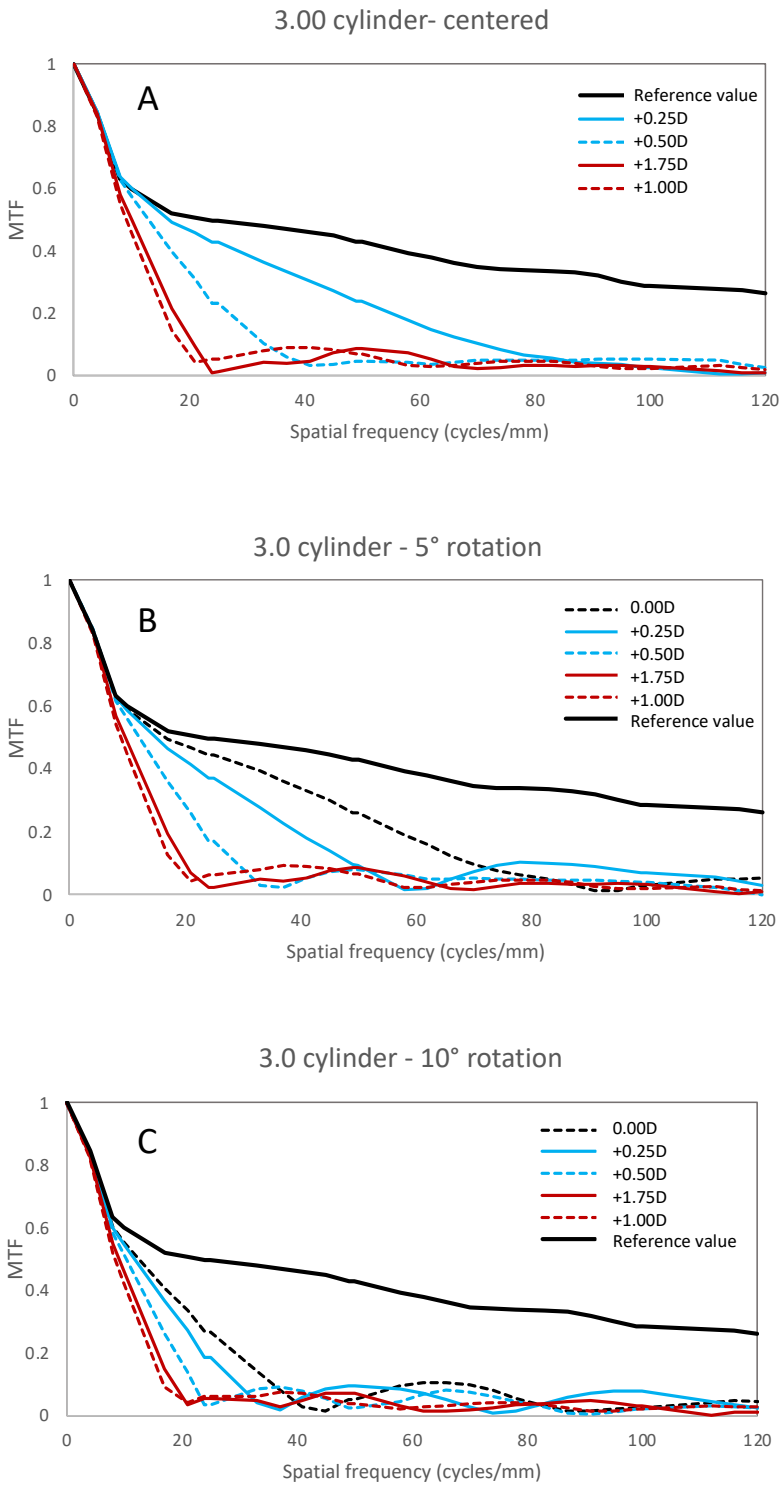


Figure 24. *MTF curves for the moderate-high cylinder (3.0D) toric IOL at different vergences (0.00 to +1.00) and at different positions: centred* ***(A)****, 5° rotated* ***(B)*** *and 10° rotated* ***(C).***

Some authors have analysed the same IOL but in a clinical setting, and obtained satisfactory uncorrected visual results across all distances (153). The optical bench used in this study allows the objective analysis of the optical quality of an IOL independently of factors such as pupil sizes, aberrations, and misalignments, which is not possible to analyse in clinical studies.

As can be observed in Table 12 and in Figures 23 and 24, the optical quality of the toric trifocal IOL analysed experienced a decrease for both cylindric powers when both rotation and defocus are induced, and this decrease becomes higher as both factors increase. The effect of defocus and the effect of rotation can be analysed reading Table 12 vertically and horizontally, respectively. The MTF value for the centred position and 0.00 D defocus, indicated with an asterisk, was considered the reference value to make comparisons with the rest of the positions. As previously mentioned, a deterioration of the MTF of more than 10 MTF units was considered as the limit of tolerable optical quality loss according to previous research (148), which correlates with a loss of almost two lines of VA.

When a low defocus (0.25D) and a low rotation (5°) are induced separately, the MTF average value stays within tolerable optical quality limits (less than 10 MTF units of loss), but any other combination exceeds that limit and leads to a significant and severe decrease (more than 10 MTF units of loss). For a centred IOL, defocus of 0.50D generates a decrease in optical quality. Moreover, when low defocus (0.25D) and a low rotation (5°) are induced at the same time, the optical quality also experiences a decrease. These findings highlight the importance of intraoperative lens position and extremely accurate power calculation.

As previously stated, these outcomes can serve as a guide for surgeons in the clinical practice given the correlation between IOL's optical quality measured with the optical bench and patient's VA (155). For example, considering the 3.00D cylinder IOL with a defocus of 0.25D and 10° rotation, a reorientation of the lens to a centred position will improve the optical

quality (and potentially the visual quality). On the other hand, for the next defocus step (i.e., 0.50D) only a reorientation will not be enough to restore the optical quality. Therefore, a correction of the residual error would be necessary, for instance, through corneal refractive surgery procedures (156).

In conclusion, the outcomes of this study suggest that there is some tolerance to rotation up to 5° and defocus up to 0.25D, separately. Combinations beyond those limits are considered detrimental to the optical quality and, therefore, might likely affect the visual performance of the toric trifocal IOL analysed.

To the best of the authors' knowledge, this is the first report on the effect of the combinations of defocus and rotation on the optical performance of this trifocal toric IOL. Thus, no direct comparison with other studies can be made. Future studies should be performed to further analyse this trifocal toric IOL with the effect of different pupil apertures and at different distances (multifocality), as well as analyse different commercially available IOLs.

2. 4. STUDY IV. Changes in optical quality induced by tilt and decentration of a trifocal IOL and a novel EDOF IOL in eyes with corneal myopic ablations.

2.4.1. Introduction

Currently, trifocal IOLs have become widely used thanks to the advantages over monofocal and bifocal IOLs in terms of vision at different distances. Trifocal IOLs overcome the spectacle dependence of monofocal IOLs and compensate for the gap between distance and near of bifocal IOLs by three differentiated peaks of vision (143), providing a more satisfactory intermediate vision (66).

Recently, a new design of IOLs has been introduced into the market to optimize the interactions between high order SA and diffractive patterns. EDOF technology is based on the creation of a single-elongated focal point to enhance the range of distances with good VA (60,157). EDOF IOLs have been analysed in both optical bench (optical quality) (157–159) and clinical (visual function) (160,161) settings, obtaining satisfactory vision over a larger range of distances and maintaining few photic symptoms and high levels of patient satisfaction.

On the other hand, more and more people reaching presbyopia who underwent ablation surgery in the past are now in need of cataract surgery and IOL implantation. In this field, myopic ablation is considered one of the most commonly conducted corneal refractive procedures, which leaves the cornea with an induced positive SA (162). In particular, lens calculation becomes more challenging due to the change in the cornea layout (67).

Additionally, it is known that lens misalignments, either decentration or tilt, will affect the optical performance of monofocal IOLs, and even more in the case of presbyopia-correcting IOLs. Decentration will change the normal light distribution of the different foci of these IOLs

(71), reducing visual performance and inducing photic phenomena (70). On the other hand, tilt will also worsen lens performance, even more in these specific lens designs (73).

Given the high prevalence of this type of patient in clinical practice, special attention should be paid to these matters. Additionally, these patients commonly have higher expectations regarding spectacle independence, which makes the final result even more important in terms of accuracy. Accordingly, the use of the optical bench allows the objective analysis of the optical quality of an IOL independent of unpredictable factors, as well as being a non-invasive and more reproducible technique than clinical studies.

Therefore, the aim of this study was to objectively analyse and compare the effect of tilt and decentration on the optical quality of a trifocal IOL and an EDOF IOL design in eyes with and without a previous corneal myopic procedure.

2.4.2. Methodology

- **IOLs analysed**

Two different IOL designs were analysed: a novel EDOF design and a conventional trifocal design. The EDOF IOL was the novel hydrophilic XACT Mono-EDOF ME4 (Santen Pharmaceutical Co Ltd), with a biconvex design and a C-loop platform. A negative Zernike 4^{th} order SA of -0.17 µm was induced for a 6-mm pupil by the diffractive rings in the anterior surface and the posterior asphericity. This IOL has an overall diameter of 12.5 mm and an optical zone of 6 mm, with the refractive rings positioned in the central 3 mm of the optical zone. These rings diffract light toward far and intermediate foci from 1.00 to 1.9 D of addition power.

The other lens evaluated was the trifocal hydrophilic FineVision IOL (PhysIOL), with an apodised diffractive trifocal design. It shows a biconvex-aspheric design with a posterior asphericity of -0.11 µm for a 6-mm pupil. Two bifocal diffractive patterns are combined for far/near and far/intermediate vision (143,149,163,164). Additionally, it shows a total diameter of 10.75 mm and an optic zone diameter of 6.15 mm, and presents an intermediate and near addition powers of +1.50 and +3.50 D.

Both IOLs analysed had a base power of 18.50 D, and an integrated ultraviolet and blue-light blocker.

- **Measurements**

- Modulation transfer function (MTF)

The PMTF optical bench (LAMBDA-X) was used to assess the image quality of the lenses at various frequencies and at different focal planes (through-focus). The device has the software version 1.13.6 and complies with International Standard Organization 11979-2 and 11979-9 requirements. For each measurement, the device was calibrated to ensure optical precision.

Through-focus MTF curves were calculated at 11 different focal points with 0.50 D steps for a discrete spatial frequency of 50 cycles per millimetre (143,150,151), which corresponds to a 0.5 Snellen VA in white light (30 cpd). Higher MTF values are associated with a better optical quality of the IOL at that focal point.

Through-focus MTF curves were calculated for each IOL and for different simulated situations/misalignments:

⇒ Induced myopic ablation: a positive +0.29 µm SA increment for 4.5 mm of pupil aperture was induced to simulate a low-moderate myopic ablation (165).

⇒ Induced lens misalignment: a 0.4 mm vertical decentration and a 4 degrees tilt were induced to simulate real IOL misalignment.

Through-focus MTF curves were calculated for the centred position and for both misalignments (vertical decentration and tilt). Therefore, a total of 6 MTF curves were assessed for each lens (no ablation + centred/decentred/tilted; myopic ablation + centred/decentred/tilted)

2.4.3. Results

Figure 25 and Figure 26 show the through-focus MTF curves for each lens (EDOF and trifocal IOLs, respectively). Figures 25A and 26A represent each IOL itself (no ablation), whereas Figures 25B and 26B represent each IOL with induced myopic ablation. Blue, black, and red lines indicate the centred, decentred, and tilted positions, respectively.

- EDOF IOL

For the centred EDOF lens (blue line), the MTF curve shows a far peak at 0.0 D and an intermediate peak at -1.00/-1.50 D approximately, which are significantly overlapped (Figure 25A). When the lens is decentred (black line) and tilted (red line), the far peak experiences a

negative shift of approximately -0.50 D and a decrease in optical quality. The intermediate peak for the centred position has a negative shift to a new near peak, and both peaks are now less overlapped.

When a positive SA was induced to simulate a myopic ablation (Figure 25B), the EDOF lens for the centred position (blue) showed a negative shift of -0.50 D for the far peak and a drop of the intermediate peak. For the decentred situation (black), an overall negative shift of -1.00 D was induced, with two differentiated peaks. For the tilted situation (red), an overall negative shift of -1.50 D was induced, generating two differentiated peaks for intermediate and near distances.

- Trifocal IOL

The trifocal lens itself (no induced SA) showed three differentiated peaks for far, intermediate and near vision, with no significant change when decentration and tilt were induced, showing a noticeable tolerance to misalignments (Figure 26A).

When a positive SA was induced to simulate a myopic ablation (Figure 26B), the trifocal lens maintained three differentiated peaks of vision but experienced a significant optical quality decrease compared to no SA. The centred position (blue) experienced a -0.50 D shift, whereas the decentred (black) and tilted (red) positions showed a -1.00 D shift, with peaks positioned in nearer object vergences.

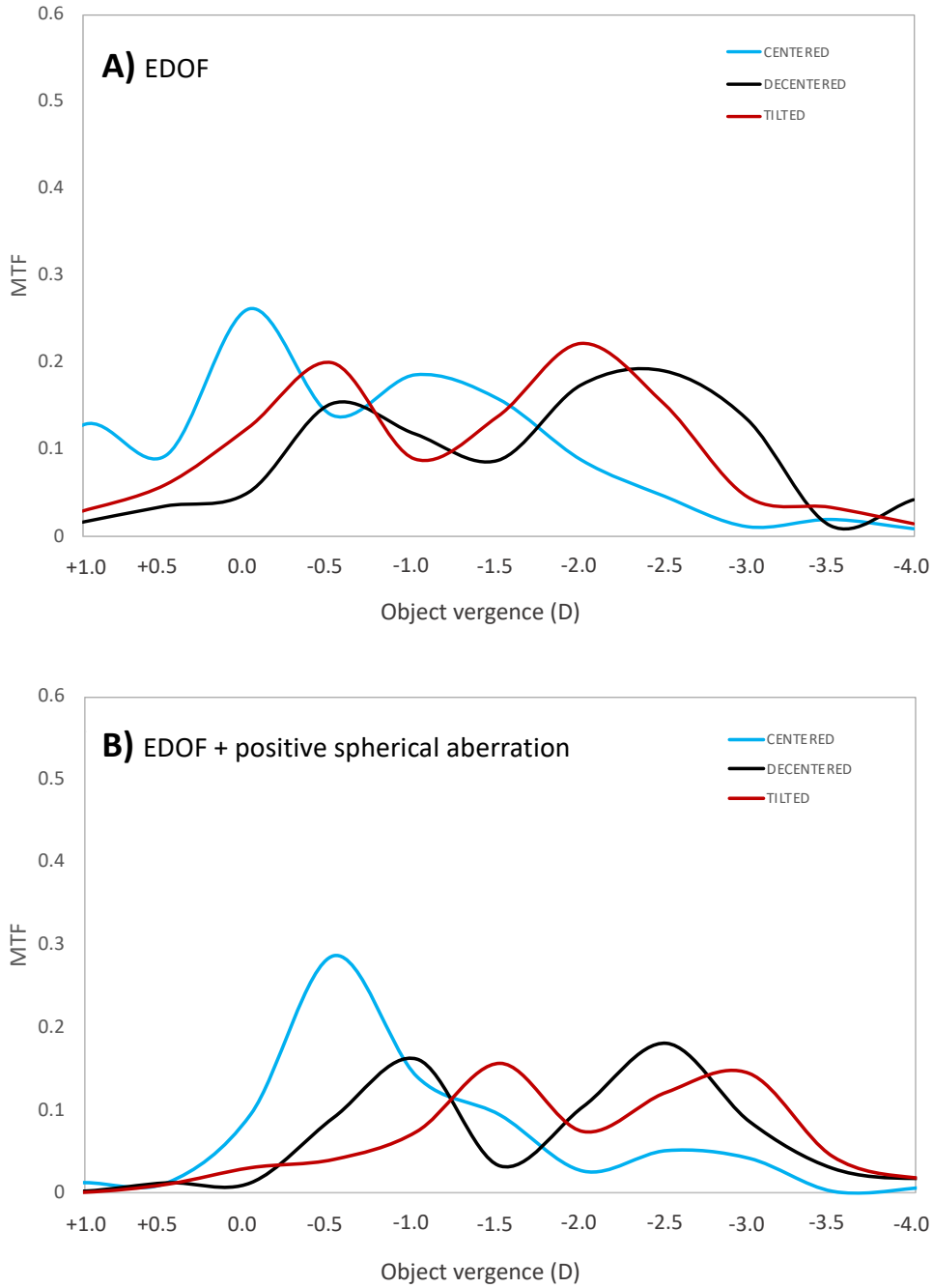


Figure 25*. Through-focus modulation transfer function (MTF) curves for EDOF IOL itself (A) and with induced positive spherical aberrations to simulate a corneal myopic ablation. Three different positions are showed: centred (blue line), decentred (black line) and tilted (red line).*

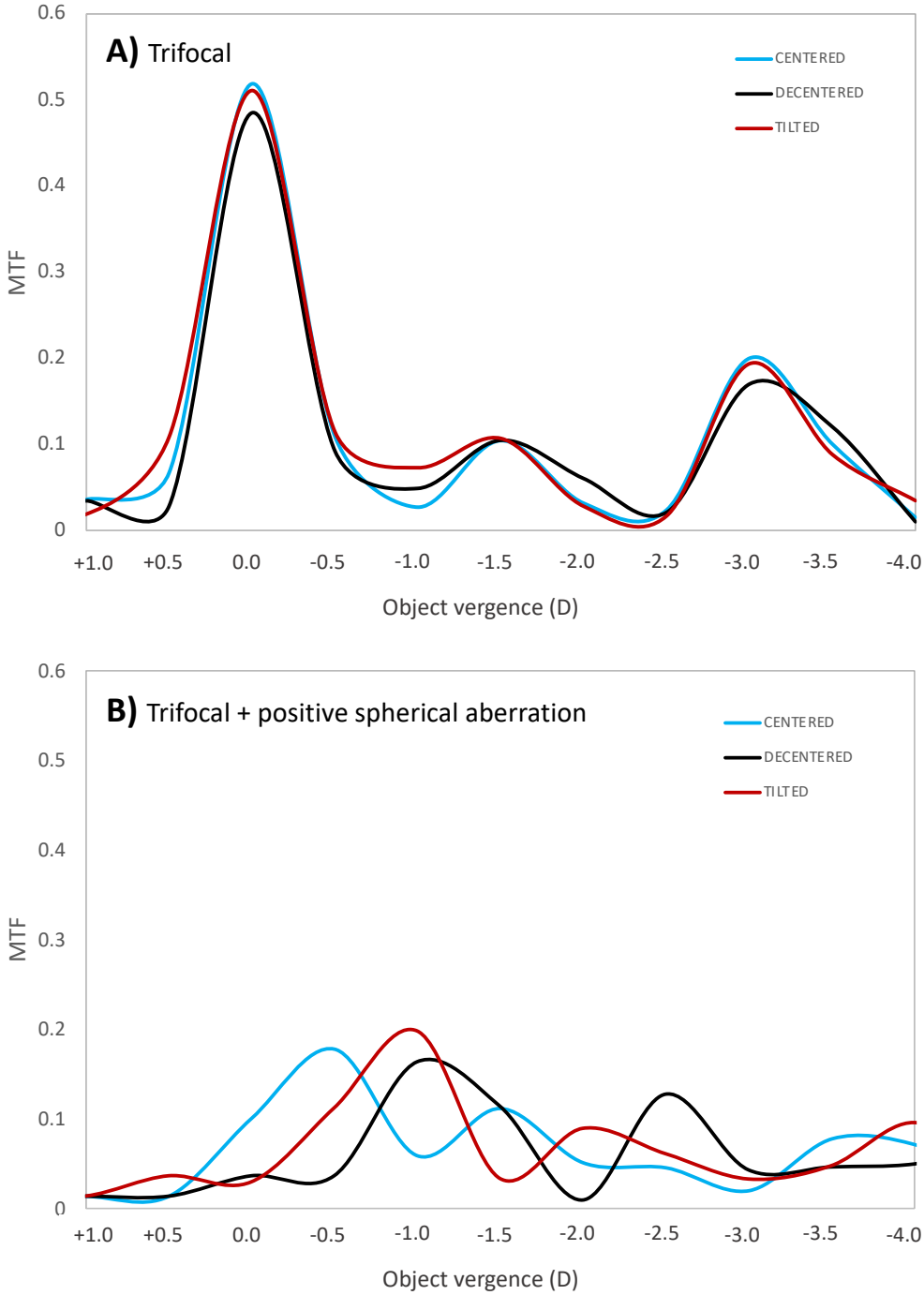


Figure 26. *Through-focus modulation transfer function (MTF) curves for the trifocal IOL (FineVision) itself (A) and with induced positive spherical aberrations to simulate a corneal myopic ablation. Three different positions are showed: centred (blue line), decentred (black line) and tilted (red line).*

2.4.4. Discussion

As stated in the previous study (Study III), the use of the optical bench allows the objective analysis of the optical quality of an IOL independent of unpredictable factors, as well as being a non-invasive and more reproducible technique than clinical studies.

Regarding the EDOF results, some studies analysing similar EDOF designs also observed more like a bifocal performance, presenting two peaks for far and intermediate vision (166,167) (see Figure 25). The -0.50 D shift induced by decentration and tilt decreased distance quality of vision to provide better performance at nearer distances.

On the other hand, the trifocal IOL performed the classic three peaks of vision corresponding to far and intermediate and near addition powers of +1.50 and +3.50 D. As reported by other researchers, these three differentiated peaks provide good visual quality at the previously mentioned distances (153). The introduction of decentration or tilt did not change or decrease visual quality at any peak of vision, showing a robust tolerance to these scenarios. In addition, these outcomes are better in comparison to those of EDOF IOL. This could be explained by the lower amount of negative SA of the trifocal IOL, which could provide the lens with higher tolerance to misalignments, besides a lower compensation of positive corneal SA (168).

Additionally, patients with previous corneal myopic ablation will present an increased amount of positive SA (162,169,170). Considering the high rate of patients who underwent corneal refractive surgery in the past, and given that the asphericity of IOL designs is calculated based on the SA of virgin corneas (i.e., no corneal refractive surgery), it is extremely useful to analyse the performance of multifocal IOL in this scenario. For that aim, both IOLs were analysed after inducing a positive SA of +0.29 µm to simulate a myopic corneal ablation.

EDOF IOL in the centred position with induced positive SA showed better performance at -0.50 D and a slight decrease in the intermediate peak quality (see Figure 25B). This myopization should be taken into consideration for cases with previous myopic ablation regarding IOL power calculation (171,172). After inducing decentration and tilt, the far and intermediate peaks of vision were maintained, but shifted to a more myopic vergence. Tilt induced a higher negative shift, changing the far and intermediate peaks for intermediate and near vergences, respectively. Thus, there would be an improvement in intermediate and near vision, with the consequent decrease at far distances. The higher negative shift of -1.00 D highlights the importance of achieving proper positioning of the IOL, even more in post-ablation patients (173).

Regarding the trifocal IOL after inducing a positive SA (Figure 25B), the three peaks were maintained but experienced a myopic shift of -0.50 D, similar to what happened with the EDOF lens. These outcomes are in agreement with those obtained by Chow et al. (172). In this case it is also important to take into account this myopization regarding IOL power calculation. Additionally, an overall shift of -1.00 D was induced when the lens was tilted and decentred, suggesting an alignment to properly recover three peaks of vision. As in the case of the EDOF IOL, the combination of both positive SA and either decentration or tilt decreases the optical quality and performance of the trifocal IOL.

In summary, the EDOF lens showed more robust optical quality tolerance in terms of induced positive SA. However, in the presence of misalignments such as tilt or decentration, it experiences a myopic shift, which is even higher in the case of previous myopic ablation. On the contrary, the trifocal IOL showed highly robust results in the presence of misalignments and better optical quality compared to the EDOF lens. However, in the presence of a myopic corneal ablation, the trifocal lens showed a decreased optical quality peak at far distances and a myopic shift. These findings agree with those obtained by Park et al. (174) who recently

evaluated the optical performance of 4 different types of IOLs (monofocal, bifocal, trifocal and EDOF) under different SA conditions. The trifocal lens was the most sensitive and the EDOF lens the least sensitive to induced SA. These results, together with the outcomes obtained in the current study might serve as a useful tool for IOL selection in the surgical practice.

All these findings emphasize the importance of accurate IOL implantation to minimize misalignments, even more in the case of post-corneal myopic ablation. It should be noted that the results obtained in this study cannot be directly extrapolated to visual performance, but they can be extremely helpful for surgeons encountering clinical situations like those described herein. It would be of interest to further correlate these results with those in the clinical practice, and to assess different amounts of SA.

CHAPTER 3.

MOLECULAR IMPLICATIONS OF PRESBYOPIA

Besides the clinical implications of age in the eye (presbyopia), attention should be paid to other ocular levels. Chapter 2 has focused on the optical correction of presbyopia. In Chapter 3 we propose to take the molecular level into consideration.

Accordingly, the expression of inflammatory biomarkers at the presbyopic ocular surface and the correlation between these biomarkers' levels and clinical parameters are assessed. The findings suggest that some of these inflammatory biomarkers could be frailty biomarkers, promoting age-related eye disease development. Knowing that they also modulate neutrophil migration and the NETosis process, a literature review regarding the link between neutrophils and their NETs and the pathogenesis of age-related eye diseases is presented.

Furthermore, since a significant proportion of the presbyopic population will seek eye health care to correct presbyopic visual symptoms, we propose presbyopia as an outstanding opportunity for detecting those changes that promote frailty and ultimately lead to age-related diseases.

3.1. STUDY V. Impact of contact lens wear on NLRP3 gene expression: implications for ocular frailty in middle-aged adults.

3.1.1. Introduction

It is known that in the 4th decade of life a wave of proteomic changes takes place in the body, and that the regulatory body systems are in decline (85). These changes are related to imbalances between pro-inflammatory and anti-inflammatory cytokines, with a tendency toward the pro-inflammatory phenotype (87). In particular, the ocular system is also involved in those age-related changes, corresponding with the onset of presbyopia (76). Here arises the term frailty as a non-specific state of vulnerability, which has been recently introduced into the ocular field as ocular frailty (89). Additionally, it is known that inflammation is a common process in all ageing, frailty and age-related diseases, and that frailty is a predisposing factor to the latter (88).

On the other hand, CLs are an external factor considered to induce inflammation in the ocular surface environment (86). In this context, ocular frailty creates a predisposition for inflammation even before CL fitting. Therefore, it is reasonable to think that CL wear could have an impact on ocular frailty, since they can trigger and extend an inflammatory process. The inflammation characteristic of the ageing process itself, along with the additional inflammation induced by CL wear is an important issue to address.

Therefore, the aim of this study was to analyse the effect of long-term CL wear on the levels of genes linked to inflammation and to evaluate potential correlations between these levels and CL-related clinical changes of the ocular surface in middle-aged adults.

3.1.2. Methodology

- **Study protocol and participants**

A total of 26 participants were enrolled in this pilot study. The total sample was grouped into CL wearers (CLW) and non-CL wearers (NCLW). All participants enrolled signed the informed consent (Annex III) prior to study procedures. The study conformed to the principles of the Declaration of Helsinki and obtained ethical approval from the Ethics Committee of San Carlos University Hospital (Madrid, Spain) (Annex I). Inclusion criteria included age between 45 and 65 years, no history of ocular allergy, ocular or systemic or inflammatory disorders or medication known to affect the ocular surface or CL wear, and no previous refractive surgery. A complete eye examination and anamnesis were performed. CLW were included in the study only if they had been wearing soft CLs for at least 1 year.

The study measurements were divided into three main sections: symptomatology, clinical assessments and molecular analysis. All measurements were performed at baseline with no CL on the eye and in that order to avoid the potential effect of the molecular tests on the clinical tests.

- **Measurements**
    - Symptomatology

OSDI questionnaire was performed as previously described in Study II (Chapter 2, Study II, Methodology).

- Clinical assessments

Tear meniscus height (TMH), ocular redness and non-invasive keratograph break-up times (NIKBUT-first and NIKBUT-average) were performed as previously described in Study I (Chapter 2, Study I, Methodology).

Corneal and conjunctival staining gradation is described in Study II (Chapter 2, Study II, Methodology)

- Molecular analysis

A conjunctival specimen was obtained from each subject by impression cytology using the EYEPRIM device (OPIA Technologies, Paris, France) (see Figure 27) and preserved in RNAlater solution (Thermo Fisher Scientific) overnight. After removing the RNAlater, conjunctival specimens were stored at -80ºC until total ribonucleic acid (RNA) isolation.

Total RNA isolation was performed with the RNeasy Mini Kit (Qiagen, Madrid, Spain) according to the manufacturer's instructions. 22 µl of total RNA were used for first-strand complementary deoxyribonucleic acid (cDNA) synthesis with the High Capacity cDNA Reverse Transcription Kit and random hexamer primers (Thermo Fisher Scientific, Madrid, Spain).

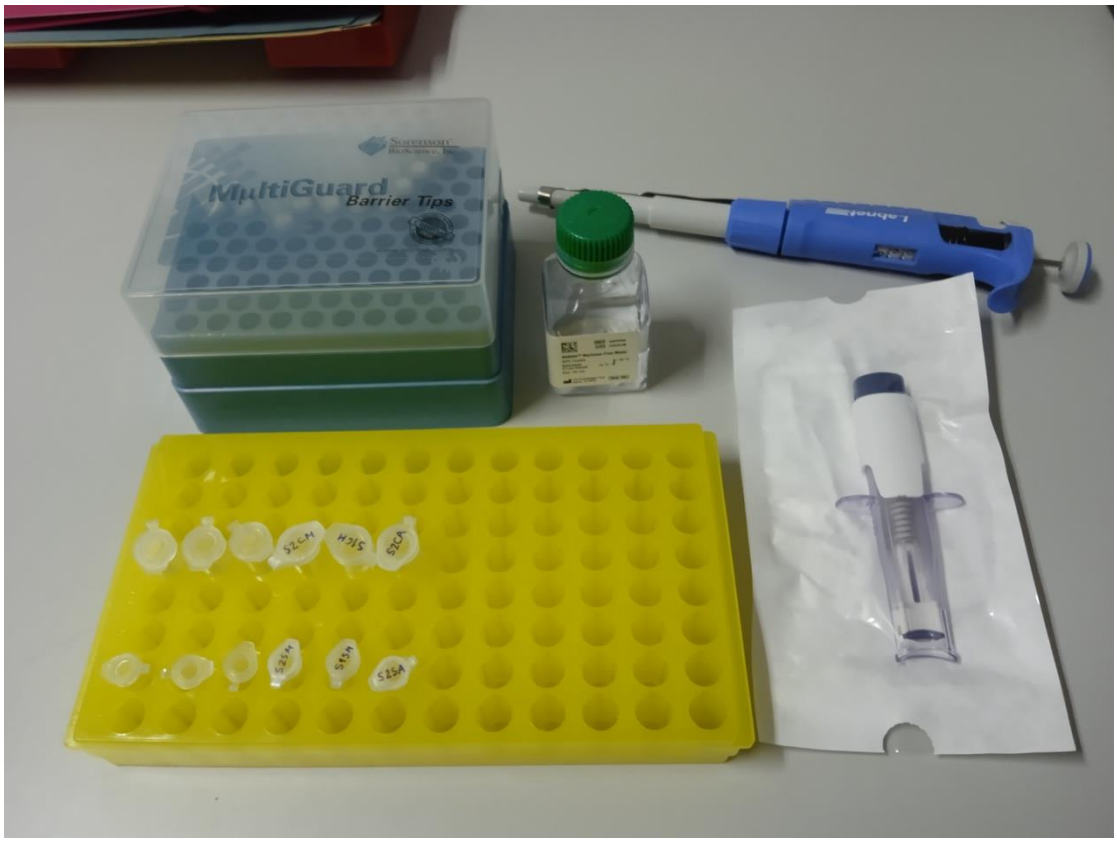


Figure 27. *EYEPRIM device to obtain conjunctival impression cytology samples (right bottom corner) and laboratory equipment.*

cDNA, gene-specific Polymerase Chain Reaction (PCR) primers (see Table 13) and the Quantitative SYBR Green Kit (Qiagen) were used to perform quantitative PCR (qPCR) in technical triplicates on an ABI Prism 7300 PCR System (Thermo Fisher Scientific). The thermal cycler was programmed for 15 minutes at 95 ºC, followed by 40 cycles of 15 s at 94 ºC, 30 s at 55 ºC and 34 s at 72 ºC (data collection step). After validation of qPCR and the internal control gene by the $2^{-\Delta Ct}$ method (175), HPRT1 gene was selected as a control to normalise mRNA relative expression.

Table 13. *Gene-specific primers used for qPCR.*

Gene	Gene-specific PCR primers	Company
HPRT1	QuantiTect Primer Assay QT00059066	Qiagen (Madrid, Spain)
IL-6	QuantiTect Primer Assay QT00083720	Qiagen (Madrid, Spain)
NLRP3	QuantiTect Primer Assay QT00029771	Qiagen (Madrid, Spain)
NK1R	5′-CTACTACTCAACCACAGAGAC-3′/ 5′-CACAGATGTGGTACACTTTC-3′	Merck (Madrid, Spain)
CD73	QuantiTect Primer Assay QT02451512	Qiagen (Madrid, Spain)
MUC16	QuantiTect Primer Assay QT01192996	Qiagen (Madrid, Spain)
TRPV1	QuantiTect Primer Assay QT00046109	Qiagen (Madrid, Spain)

- **Statistical analysis**

The statistical analysis was performed with the IBM SPSS statistics software version 25.0 (SPSS Inc., Chicago, IL., USA). For the analysis of quantitative data (clinical and molecular parameters) the Shapiro-Wilk and Mann-Whitney tests were used. For the analysis of qualitative data (staining values) the chi-square test was used. Kendall's Tau-b coefficient (τb) was used for the analysis of correlations between clinical and molecular data. Data is expressed as mean ± standard deviation. Statistical significance was set at 95% ($p<0.05$).

3.1.3. Results

A total sample of 26 individuals was recruited. The NCLW group included 12 individuals (8 females and 4 males) with a mean age of 53.8 ± 7.0 years, whereas the CLW group included 14 individuals (9 females and 5 males) with a mean age of 53.9 ± 4.1 years.

No significant changes were observed in any clinical parameter between CLW and NCLW groups (p>0.05 all, see Table 14), showing no clinical alteration in the ocular surface induced by CL wear.

***Table 14**. Clinical parameters for contact lens wearers (CLW) and non-contact lens wearers (NCLW).*

	NCLW	CLW	p-value
TMH (mm)	0.30 ± 0.16	0.31 ± 0.07	0.50
Bulbar redness	1.40 ± 0.36	1.33 ± 0.30	0.50
NIKBUT (sec)	10.63 ± 6.86	10.01 ± 5.59	0.98
NIKBUT-avg (sec)	13.73 ± 6.48	12.98 ± 5.08	0.65
Corneal staining	0.27 ± 0.65	0.00 ± 0.00	0.20
UBC staining	0.00 ± 0.00	0.08 ± 0.28	1.00
LBC staining	0.09 ± 0.30	0.08 ± 0.28	1.00
NBC staining	0.73 ± 0.79	0.69 ± 0.75	1.00
TBC staining	0.45 ± 0.52	0.23 ± 0.60	0.06
Tarsal staining	0.18 ± 0.40	0.15 ± 0.38	1.00
OSDI	15.94 ± 9.06	13.19 ± 9.70	0.46

*TMH: tear meniscus height; NIKBUT: non-invasive keratograph break-up time (first break-up); NIKBUT-avg: average of all tear break-up times; UBC: upper bulbar conjunctival; LBC: lower bulbar conjunctival; NBC: nasal bulbar conjunctival; TBC: temporal bulbar conjunctival; OSDI: Ocular Surface Disease Index questionnaire. * indicates statistical significance between groups. Data expressed as mean ± standard deviation. Staining scores recorded with the Efron scale.*

Molecular data is summarised in Table 15. Given that HPRT1 expression was not affected by CL wear, this gene was selected as a control. IL-6, NK1R, and TRPV1 showed no significant difference between groups ($p>0.05$ all). NRLP3 and MUC16 genes were overexpressed in CLW (median 2.6-fold and 3.5-fold higher than NCLW, respectively) (see Figure 28A and 28B). On the other hand, CD73 gene expression showed a significant down-regulation in CLW compared to NCLW (median 6.4-fold lower) (see Figure 28C).

Table 15. *Molecular parameters for contact lens wearers (CLW) and non-contact lens wearers (NCLW).*

	NCLW	**CLW**	**p-value**
HPRT1 (control)	1.09 ± 0.46	1.10 ± 0.94	0.43
IL-6	0.07 ± 0.10	0.11 ± 0.16	0.35
NLRP3	0.01 ± 0.01	0.05 ± 0.08	**0.02***
NK1R	0.00 ± 0.00	0.00 ± 0.00	0.46
CD73	1.72 ± 0.85	0.86 ± 1.08	**0.02***
MUC16	0.50 ± 0.28	1.78 ± 1.52	**0.04***
TRPV1	0.24 ± 0,08	0.29 ± 0.20	0.71

** indicates statistical significance between groups. Data expressed as mean ± standard deviation of mRNA fold change.*

Additionally, correlations between clinical and molecular data were analysed (see Table 16). Only the genes with significant differences in its expression caused by CL wear were evaluated (i.e., NRLP3, CD73 and MUC16). A significant negative correlation was found between MUC16 mRNA levels and tarsal conjunctival staining, being inversely proportional ($\tau b = - 0.624$; $p = 0.040$). The rest of the clinical and molecular parameters did not correlate with each other ($p>0.05$ all).

Table 16. *Correlations between clinical data and the genes experiencing significant differences in its expression caused by CL wear in CLW.*

	NLRP3		**CD73**		**MUC16**	
	τb	**p-value**	**τb**	**p-value**	**τb**	**p-value**
TMH	-0.256	0.222	-0.055	0.815	-0.500	0.061
Bulbar redness	0.160	0.457	-0.405	0.093	0.261	0.338
NIKBUT	-0.026	0.903	-0.055	0.815	-0.389	0.144
NIKBUT-avg	-0.128	0.542	-0.018	0.938	-0.389	0.144
Corneal staining	- [a]	- [a]	- [a]	- [a]	- [a]	- [a]
UBC staining	0.196	0.423	0.341	0.206	0.236	0.439
LBC staining	0.196	0.423	0.341	0.206	0.236	0.439
NBC staining	-0.220	0.348	0.225	0.384	0.298	0.312
TBC staining	0.212	0.377	0.340	0.198	0.215	0.469
Tarsal staining	0.097	0.693	-0.318	0.239	-0.624	**0.040***
OSDI	0.026	0.902	-0.321	0.179	-0.177	0.521

CLW: contact lens wearers; TMH: tear meniscus height; NIKBUT: non-invasive keratograph break-up time (first break-up); NIKBUT-avg: average of all tear break-up times; UBC: upper bulbar conjunctival; LBC: lower bulbar conjunctival; NBC: nasal bulbar conjunctival; TBC: temporal bulbar conjunctival; OSDI: Ocular Surface Disease Index questionnaire.
[a.]: not possible to calculate. At least one of the variables was constant.

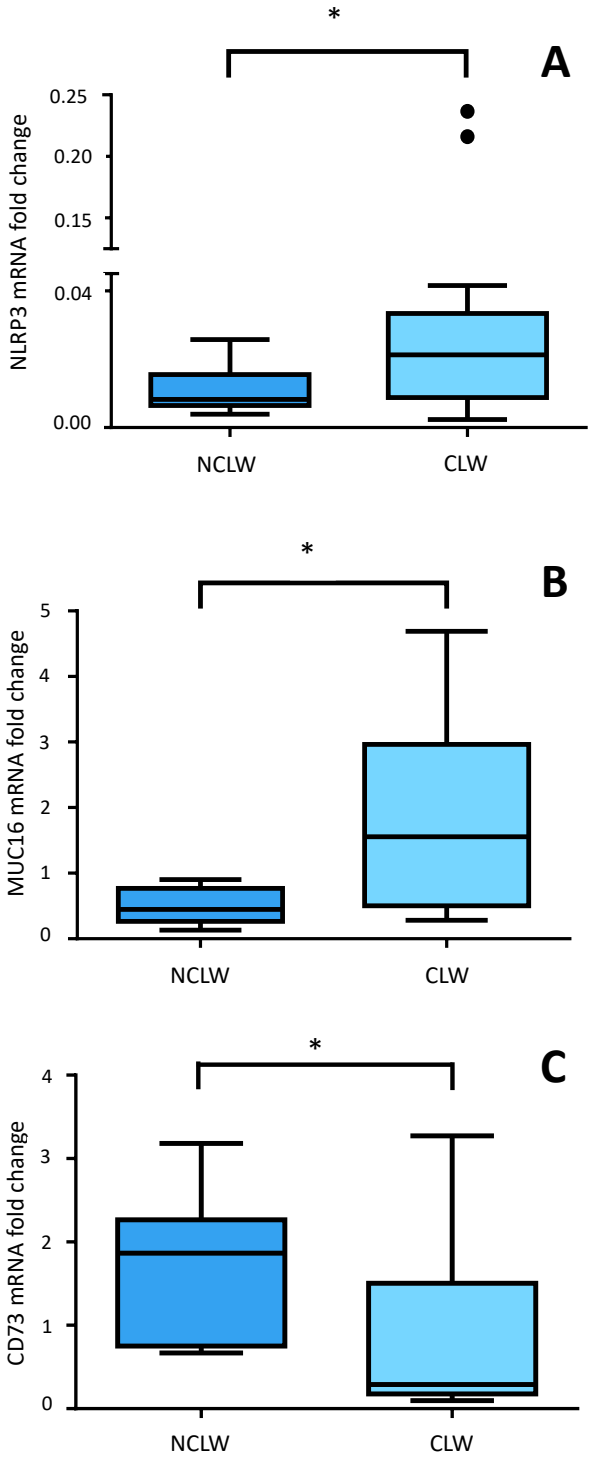


***Figure 28**. NLRP3 (A), MUC16 (B) and CD73 (C) gene expression for CLW and NCLW in conjunctival cells. HRPT1 signal was used to normalised gene expression level. The lower box line represents the 25th percentile, the middle line the median value, and the upper line the 75th percentile. Whiskers indicate extreme values and dots represent outlier values. * indicates statistical significance between groups ($p<0.05$).*

3.1.4. Discussion

Among the molecules linked to inflammation analysed in this study, the ones which saw their expression affected by CL wear were NLRP3, CD73 and MUC16. NLRP3 and MUC16 were overexpressed in CLW, whereas CD73 showed a reduced expression compared to NCLW.

Inflammasomes play a key role in molecular inflammation and are activated by several factors, such as infections with bacteria, virus or fungi, among others. Its activation usually leads to inflammation resolution, but at the same time, can be partly responsible for the inflammatory process itself (176). In particular, nod-like receptor protein 3 (NLRP3) inflammasome is one of the most studied NLRs. Elevated levels of NLRP3 have been found in pterygium (177), bacterial infection (178) and in dry eye patients, contributing to the onset and development of the inflammatory process in the latter (179). In the current study, NLRP3 mRNA levels were increased, showing an overexpression of this inflammasome in CLW. Additionally, Prince et al. showed a race-specific alteration of IL1-β mRNA in blood cells in middle-aged adults with frailty, a major initiator and promoter of inflammation and an effector of NLRP3 inflammasome (180).

Regarding CD73 protein, lower mRNA levels were found in CLW compared to NCLW, showing a reduced expression. CD73, also known as ecto-5′-nucleotidase, is a major nucleotide metabolizing enzyme that regulates immunity and inflammation (181). In combination with CD39 (NTPDase 1), CD73 degrades adenosine triphosphate (ATP) to adenosine (ADO). ATP has a pro-inflammatory immune cell activity, whereas ADO is considered an important endogenous anti-inflammatory regulator, and its receptors are a key factor in suppressing responses to infection (182). Thus, the lower expression of CD73 will create imbalances between these two, increasing ATP levels and decreasing ADO levels. Additionally,

extracellular ATP has been found to activate NLRP3 inflammasome, therefore triggering a pro-inflammatory process (183). These outcomes suggest that long-term CL wear might increase vulnerability toward an inflammatory response.

MUC16 is a shed membrane-bound soluble mucin known to prevent damage and infection by maintaining its mucosal barrier function. Along with other mucins, MUC16 plays an important role in maintaining tear film stability and quality, and correct wettability, osmolarity and homeostasis of the ocular surface. Therefore, changes or alterations in its expression might lead to several ocular diseases (184).

Regarding the outcomes obtained in this study, MUC16 mRNA levels were found increased in CLW, showing an upregulation of this mucin. These results are in agreement with the literature. Caffery et al. found increased levels of both soluble MUC16 and MUC16 mRNA concentrations in conjunctival cells of Sjogren's Syndrome compared to controls (185). Additionally, Gipson et al. found an elevated MUC16 mRNA level in postmenopausal women with a history of non-Sjogren's dry eye (186). This upregulation of MUC16 is thought to be a compensatory mechanism for irritation and inflammation aiming to repair local areas of mucin loss (185,186). Moreover, changes in mucin concentration contribute to tear hyperosmolarity (184). This hyperosmolarity has been associated with elevated mRNA levels of NLRP3, therefore activating this inflammasome (187,188).

The results obtained suggest that there is an inflammatory process in CLW, with an overexpression of NLRP3 inflammasome and MUC16, and a reduced expression of CD73. These three inflammatory components seem to be interrelated. The higher levels of ATP caused by the underexpression of CD73 lead to NLRP3 activation. This inflammasome takes part in inducing MUC16 expression, which, at the same time, will create a hyperosmolar environment, activating the NLRP3 inflammasome (see Figure 29).

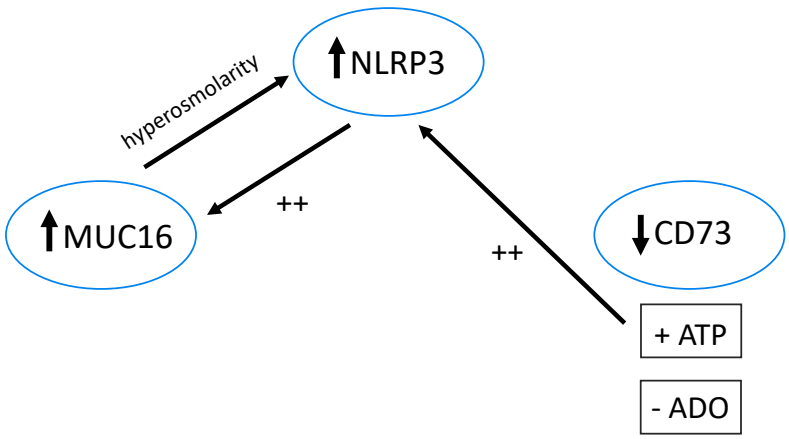


Figure 29. *Connexion among NLRP3 inflammasome and CD73 and MUC16 proteins in CLW. High levels of ATP caused by a lower expression of CD73 will activate NLRP3 inflammasome, which, at the same time, will induce MUC16 expression. Additionally, MUC16 might induce hyperosmolarity that activates NLRP3 inflammasome. ATP: adenosine triphosphate; ADO: adenosine.*

Regarding correlations between clinical and molecular parameters, only a significant negative correlation was found between conjunctival tarsal staining and MUC16 mRNA levels. This finding suggests that the expression of MUC16 in the ocular surface avoids tarsal staining. This barrier role of MUC16 has already been observed in previous investigations. Blalock et al. (189) indicated that when MUC16 release was induced, the penetrance barrier on epithelial cells was reduced. In fact, it was found that when fluorescein staining increased, so did the level of MUC16 to compensate for the damage (186). However, although it is considered an essential component of the epithelial protective barrier, an increased release of MUC16 might be damaging to the ocular surface (189).

In conclusion, the findings of this study revealed changes in the expression of several inflammatory molecules associated with soft CL wear, suggesting that long-term CL wear could be a risk factor for ocular frailty since it promotes and triggers an inflammatory response. This inflammatory process seems to be orchestrated by NLRP3 inflammasome and modulated by CD73 and MUC16 proteins, which could serve as frailty biomarkers at the ocular surface level. Given the high rate of CL prescription worldwide (190), and considering that frailty is a key factor regarding age-related diseases (88), these outcomes are of utmost importance. Additionally, clinical examination failed to detect inflammation. Therefore, a molecular analysis should complement clinical tests to detect the early stages of ocular surface inflammation.

3.2. Neutrophils and neutrophil extracellular trap components: emerging biomarkers and therapeutic targets for age-related eye diseases

3.2.1. Introduction

Neutrophils, like many structures of the body, also undergo functional changes during ageing (191,192). They are the first line of defense against pathogens by mediating innate immunity, but participate as well in adaptive immunity (193,194). The link between these two actions is known as the NETosis process (194,195).

During NETosis process, neutrophils secrete a meshwork containing nuclear/mitochondrial DNA (extracellular DNA, eDNA) and cytosolic granule peptides/proteins (196,197). This meshwork is called Neutrophil Extracellular Trap (NET) because of its function to immobilize and kill pathogens. However, the presence of NETs in the extracellular medium is known to activate and exacerbate an inflammatory reaction (193–195), a key factor in age-related diseases. Figure 30 represents a schematic diagram of these NETs and the effect their constituents might have on ocular cells.

From Study V, it can be concluded that NLRP3 and CD73 inflammation biomarkers alter their expression in long-term CL wear. This finding suggests the hypothesis that these inflammation biomarkers could serve as frailty biomarkers at the ocular surface level since they are specially sensitive to external agents such as CLs, promoting age-related eye diseases (AREDs) development (by definition, frailty predisposes to age-related disease).

On the other hand, the scientific literature shows that NLRP3 and CD73 are implicated in AREDs development (198–202) and modulate both neutrophil migration and the NETosis process (203–205). We aimed to study the possible role of neutrophils and their processes in

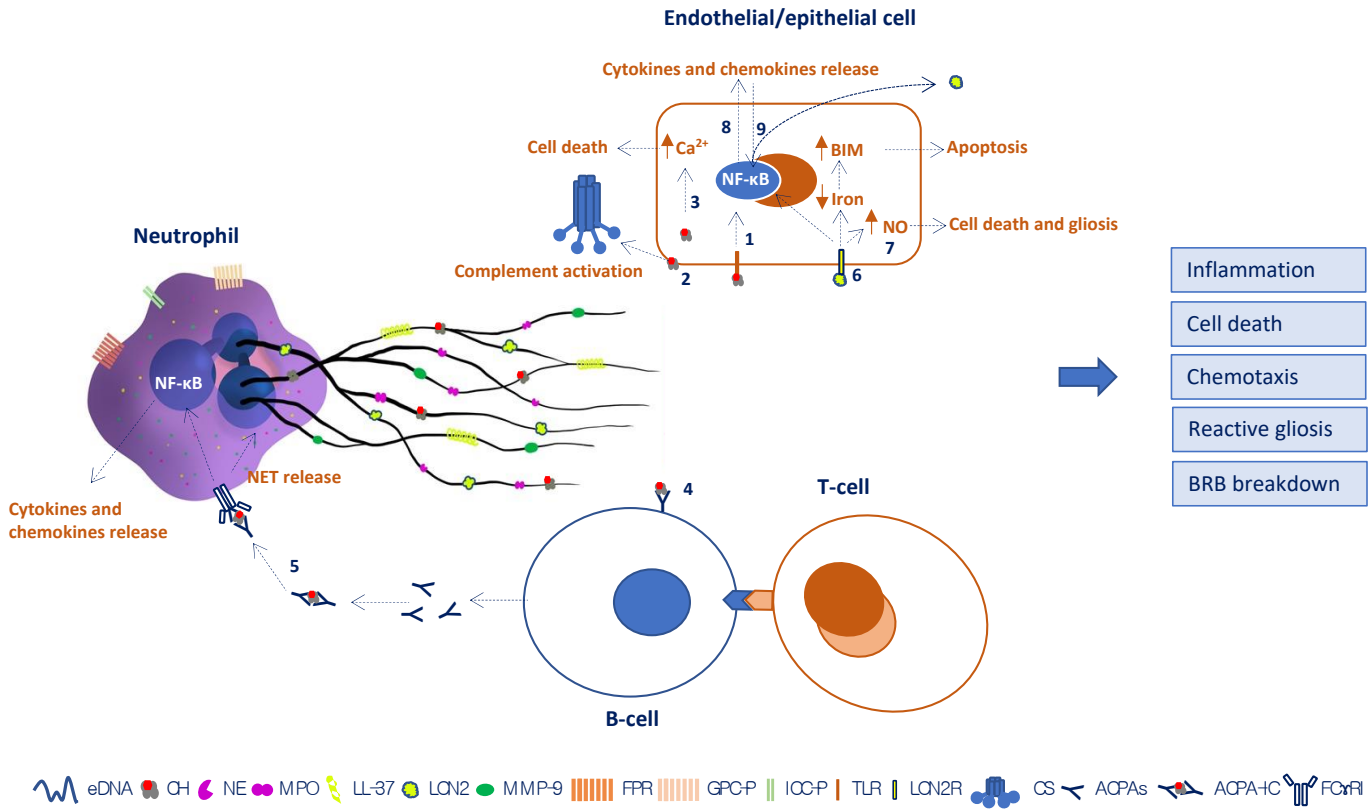


Figure 30. *Schematic representation of the Neutrophil Extracellular Trap (NET) and possible harmful effects of NET constituents on ocular cells involved in AREDs.*
The figure shows a neutrophil with some surface receptors [formyl peptide receptors (FPR), Fcɣ receptors I (FcɣRI), G protein-coupled purinergic receptors (GPC-P, e. g., P2Y2, P2Y6, A3, and A2A), and ion channel-coupled purinergic receptors (ICC-P, e.g., P2X7)] and NET components [extracellular DNA (eDNA) and components released from neutrophil primary granules (MPO, myeloperoxidase; NE, neutrophil elastase), secondary granules (LL-37, cathelicidin; LCN2, lipocalin 2), and tertiary granules (MMP-9, matrix metalloproteinase-9)]. NET components can cause inflammation, cell death, chemotaxis, reactive gliosis, and BRB breakdown on eye tissues of AREDs patients. The picture illustrates possible cell damage mechanisms evoked by NETs components in the eye: extracellular citrullinated histones (CH) activate (1) toll-like receptors (TLR), driving the NF-κB-mediated transcription of pro-inflammatory cytokines, and (2) the complement system (CS). CH can also penetrate the plasma membrane, resulting in an influx of Ca2+ that leads to cell death (3). B-cells also recognize CH and, after T-cells stimulation, make anti-citrullinated protein antibodies (ACPAs). ACPAs can form immune complexes with citrullinated proteins (ACPA-IC) (5) that activate FcɣRI of neutrophils and dendritic cells. This activation finally leads to the release of pro-inflammatory cytokines and NETs. Extracellular LCN2 binds to LCN2 receptors (LCN2R) that mediates its uptake via receptor-mediated endocytosis (6). Intracellular LCN2R can expel iron outside the cell, which induces the expression of the pro-apoptotic protein Bim. Intracellular LCN2 induces NO formation, promoting cell death and gliosis (7), and triggers the synthesis and release of cytokines and chemokines (8). Conversely, pro-inflammatory mediators evoke the synthesis and release of more LCN2 from local cells (9).

AREDs onset. If this link was demonstrated, it could indicate that NLRP3 and CD73 might be frailty mediators and that, by the modulation of neutrophils and their functions, they predispose to AREDs onset. Thus, we performed a literature review on neutrophils and NETs components.

3.2.2. Results and discussion

The results obtained from this review showed that neutrophils and NETs components are implicated in the development of the main AREDs (e.g., age-related macular degeneration, diabetic retinopathy, glaucoma and dry eye disease). Table 17 shows an overview of the link between neutrophils/NETs components and the mentioned diseases.

These AREDs have shown to experience oxidative and inflammatory molecular mechanisms, which are common to the ageing process and to other age-related diseases (206–209). The impairment of NETs formation and degradation seems to be one of these common processes, since individuals suffering from AREDs show local recruitment of neutrophils and NETs components overexpression (210,211,220–224,212–219).

In this sense, the neutrophil-to-lymphocyte ratio (NLR) parameter (the number of neutrophils divided by the number of lymphocytes in a peripheral blood sample) has been studied and validated as AREDs biomarker (225–231) since it is considered a systemic inflammation indicator (232–234) and is linked to frailty (235).

All these findings indicate that neutrophils, their NETs and their constituents could be used as AREDs diagnostic biomarkers and can be considered therapeutic targets. For instance, Lipocalin-2 (LCN2), a NET component with neuroinflammatory and vascular damage modulation functions, has been proposed as a diagnostic component for some neurodegenerative diseases, such as Alzheimer's, Parkinson's, Huntington's diseases or multiple sclerosis (236). In the same way, we have suggested that this protein could be a potential AREDs biomarker.

Table 17. Overview of the link between neutrophils/NETs components and age-related eye diseases.

DRY EYE DISEASE
• DED patients present a raised blood NLR and SII value (229,237), conjunctival neutrophil infiltration (222), and high levels of neutrophils in tears (210,221,222). • Patients with severe DED present higher levels of NETs, eDNA, NE, MPO, LCN2 and MMP-9 in tears (210–212,222,223). The ocular surface wash of these patients also contains abnormal amount of citrullinated proteins, PAD4 enzyme, and ACPAs (238). • Tear neutrophils levels correlate positively with DED severity (210,221). • Neutrophils promote meibomian gland dysfunction, the most common cause of evaporative DED (221). • Hyperosmolar stress induces NET formation. This process is inhibited by lipidic mediators like annexin-1 and lipoxin A4 that modulate the recruitment and survival of neutrophils (239). • Clinical studies support the use of DNase1, ocular surface immunoglobulin (IgG)-eye drops and some modulators of neutrophil recruitment like purinergic receptor ligands for DED treatment (238,240–242).
GLAUCOMA
• Glaucoma patients present a high blood NLR value, which correlates positively with glaucomatous damage (226,228). It has been proposed the measure of SII as inflammatory predictor in POAG patients (243). POAG patients also present neutrophil infiltration and overexpression of cytokines that promote the NETosis process in the TM (244–246). • The aqueous humour of POAG patients and retinal ganglion cells of glaucoma animal models present high levels of LCN2 (218,219,224). Some authors have suggested the biomarker/therapeutic target use of LCN2 for glaucoma (247). The optic nerve of POAG patients also presents a high level of PAD2 and citrullination (248). Müller cells of glaucomatous animals contains citrullinated filaments (249).

- Preclinical studies have demonstrated the hypotensive and neuroprotective effect of some purinergic receptor's ligands (250–256).

AGE-RELATED MACULAR DEGENERATION

- AMD patients present a high blood NLR value, which correlates positively with disease severity (226,227,257). These patients and AMD animal models present neutrophil infiltration in choroid and retina (216,217).
- Retinal neutrophils of AMD patients overexpress NWTs, NE, MPO, and LCN2 (216,217). They also overexpress LCN2 and possibly MMP-9 at the plasma level (213,214). Retinas from AMD post-mortem patients present an abnormal level of citrullination (258).
- Neutrophils compromise the oBRB (259) and secrete VEGF (260).
- LCN2 promotes neutrophils' migration and LCN2 activated neutrophils cause retinal degeneration (216,261).
- Some authors have suggested the biomarker/therapeutic target use of LCN2 for AMD (214,216,217,262). Preclinical studies support the use of regulators of the retinal LCN2 mRNA expression to treat AMD (216,217).
- Preclinical studies support the use of modulators of NET formation like P2X7 receptor-blocking agents for AMD treatment (263).

DIABETIC RETINOPATHY

- DR patients present a high blood NLR value, which correlates positively with disease severity (230,231). A DR rat model present retinal neutrophil infiltration (264).
- The retinal of diabetic rats and the vitreous fluid/blood of DR patients present abnormal levels of NETs, eDNA, NE, MPO, and LCN2 (215,220,264). Blood arginine (a precursor of citrulline) and citrulline are abnormally high in DR patients (265).
- Hyperglycaemia induces the NETosis process, from blood neutrophils of a diabetic rat model and DR patients (especially in those with PDR) (264).
- NE increases human retinal endothelial cells permeability *in vitro* and mediates retinal vascular damage in a diabetic mouse model (266). LCN2 also contributes to the retinal vascular damage in DR patients (215,267,268).

- Preclinical studies support the use of lipidic mediators and purinergic receptor ligands for DR treatment (269–273).

AMD, Age-related macular degeneration; eDNA, extracellular DNA; DED, dry eye disease; DR, Diabetic retinopathy; LCN2, lipocalin 2; MMP-9, matrix metalloproteinase-9; MPO, myeloperoxidase; NE, neutrophil elastase; NETs, neutrophil extracellular traps; NLR, neutrophil-to-lymphocyte ratio; oBRB, blood-retinal barrier; PAD4, peptidyl-arginine deiminase 4; POAG, primary open-angle glaucoma; SII, systemic immune- inflammation index; TM, trabecular meshwork; VEGF, vascular endothelial growth factor.

We have also proposed the use of ligands of neutrophil receptors (like pro-resolution mediators and purinergic ligands) as therapeutic agents against AREDs. Their function is to neutralize neutrophil recruitment, preventing NETs components to accumulate locally. These ligands can activate NLRP3 inflammasome, which, at the same time, controls neutrophils migration and NETosis processes. Likewise, these ligands are hydrolysed by CD73 ectonucleotidase (expressed by neutrophils), also controlling neutrophils migration.

These findings indicate that because CD73 and NLRP3 are neutrophils and NETosis modulators, they could be frailty mediators predisposing to AREDs (see Figure 31). Therefore, it would be of interest to study these and other frailty biomarkers on the presbyopic ocular surface. It has been shown that detecting these biomarkers at early stages could prevent or at least delay age-related disease onset. A way to do this would be via ocular surface samples, which is a less invasive method compared to others. In fact, tear film and ocular surface cells represent a powerful source of biomarkers for several diseases (274,275), which should be taken into consideration regarding future investigations.

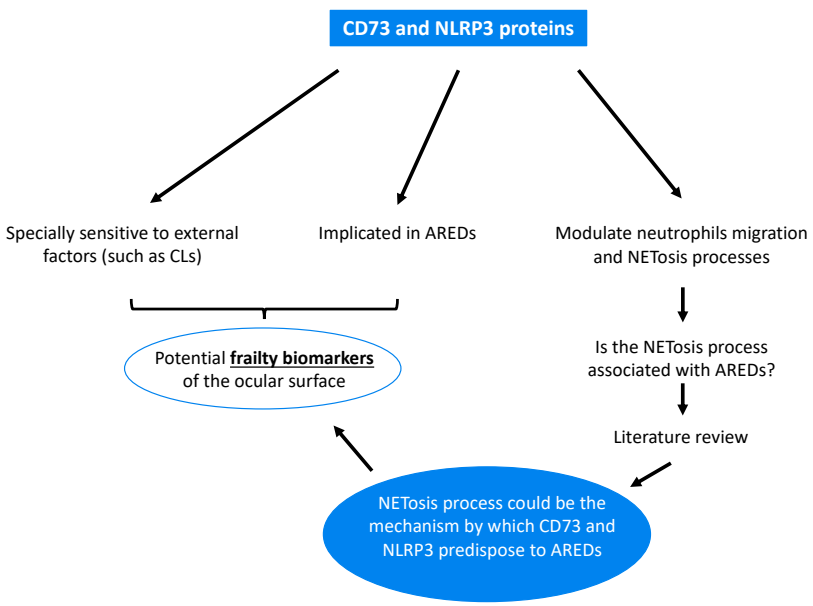


Figure 31. *Schematic diagram of the hypothesis presented in this section. CD73 and NLRP3 proteins could be frailty mediators by modulating neutrophils and NETosis processes, therefore predisposing to AREDs.*

3.3. Presbyopia: an outstanding and global opportunity for early detection of pre-frailty and frailty states

As presented in previous sections, frailty syndrome is described as “a nonspecific state of vulnerability, which reflects multisystem physiological change” (88), and it is considered a predisposing factor to age-related diseases, including AREDs (89). In fact, the molecular alterations present in frail individuals are the underlying cause of future age-related diseases. Due to population ageing and the increasing life expectancy prospects, age-related diseases, including eye diseases, are expected to grow (112). AREDs, including DED, glaucoma, AMD, and diabetic retinopathy, represent a major global health issue based on their increasing prevalence and disabling action, with enormous personal and economic consequences (276). Therefore, there is an urge to address this global eye health matter.

The findings obtained from the literature review of the previous section indicate that certain modulators of neutrophils and the NETosis process (e.g., CD73 and NLRP3) could serve as frailty biomarkers in the presbyopic ocular surface. It has been shown in recent studies that frailty signs were present in middle-aged adults (40-50 years old) (180,277,278), which coincides with presbyopia onset. Considering the poor health outcomes of frailty syndrome (multimorbidity and mortality), early detection of this affection could potentially prevent or delay these outcomes (278). However, due to the lack of signs and symptoms of this syndrome, early diagnosis is still in need of improvement.

Herein, we suggest that the unavoidable visit of presbyopic individuals to eye care professionals looking for a solution to their symptoms could be an opportunity for the early detection of frailty in a large population (see Figure 32). Data back the concept of the eye and its tear as a source of diagnostic biomarkers of ocular and systemic diseases, including age-related ones (274,279–281). Thus, it would not be surprising that tears would contain frailty biomarkers. As any eye practitioner can easily collect tears, screening for frailty biomarkers from tears of presbyopic subjects may represent an outstanding opportunity for early detection of frailty state, allowing timely intervention and thus preventing poor clinical outcomes.

It would also be useful to validate frailty biomarkers and study frailty's underlying mechanism at the ocular level, allowing the study of future biomarkers. This hypothesis has been published and its scientific basis has been accepted (282).

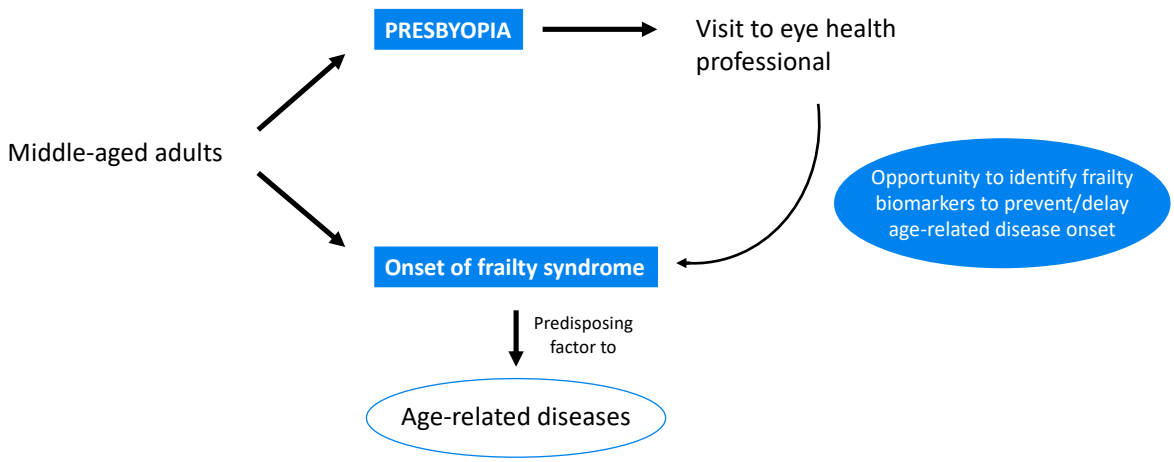


Figure 32. *Schematic overview of the idea of performing a large-scale screening of the presbyopic population to identify frailty biomarkers.*

CHAPTER 4.

Chapter 4 includes the main conclusions of this work, a proposal of future directions, and the scientific dissemination of the data provided by this thesis.

4.1. CONCLUSIONS

The following is a summary of the conclusions taken from the studies performed in this thesis:

1. Three measurements are recommended to obtain excellent repeatability results during CL wear for tear meniscus height measured with the K5M device. CLs might be considered a potential artifact affecting NIKBUT-average repeatability since it worsens with CL wear. Time of wear seems not to affect NIKBUT-average.

2. EDOF CL and the aspheric design perform similarly in terms of vision, impact on the ocular surface and symptomatology. More attention should be paid to the effect of CL wear on dry eye symptoms given the more susceptible presbyopic ocular environment.

3. Trifocal toric IOLs tolerate defocus up to 0.25D and rotations up to 5°. Exceeding those limits significantly decreases optical quality, which would likely lead to a worsening in visual quality.

4. EDOF IOLs tolerate positive spherical aberrations (previous corneal myopic ablations) better than trifocal IOLs but experience a myopic shift in the presence of misalignments (tilt or decentration). On the contrary, trifocal IOLs tolerate misalignments better than the EDOF design and obtain better optical quality, but it decreases in the presence of positive spherical aberrations.

5. Long-term soft CL wear seems to promote an inflammatory process by modifying the expression of several inflammatory biomarkers, therefore increasing ocular surface

frailty. This early stage of ocular inflammation is not detected by clinical tests, which highlights the importance of addressing presbyopia from the molecular level.

6. CD73 and NLRP3 proteins could be frailty mediators given their ability to modulate neutrophils migration and NETosis processes. Presbyopia can be an outstanding opportunity for frailty early detection, therefore preventing AREDs, by performing a large-scale tear sampling screening of frailty biomarkers.

4.2. FUTURE DIRECTIONS

The results obtained in this thesis can establish the basis for future lines of research, which are classified into two different topics according to the chapters presented herein:

Presbyopia correction:

- To investigate new CL materials or coatings to improve comfort during wear in the presbyopic population.
- To investigate new presbyopic optical designs to reduce dysphotopsias.

Ageing biomarkers:

- To identify frailty biomarkers at the ocular surface level.
- To identify ageing and successful-ageing biomarkers and to further the knowledge of clinical and molecular characteristics of the presbyopic ocular surface compared to other population groups (already in development).

4.3. SCIENTIFIC DISSEMINATION

This thesis has resulted in the following peer-reviewed publications and communications:

Scientific articles

Chapter 2:

- García-Montero M, Rico-Del-Viejo L, Lorente-Velázquez A, Martínez-Alberquilla I, Hernández-Verdejo JL, Madrid-Costa D. Repeatability of Noninvasive Keratograph 5M Measurements Associated With Contact Lens Wear. Eye Contact Lens. 2019;45(6):377-381. doi:10

- Martínez-Alberquilla I, García-Montero M, Ruiz-Alcocer J, Crooke A, Madrid-Costa D. Visual function, ocular surface integrity and symptomatology of a new extended depth-of-focus and a conventional multifocal contact lens. Cont Lens Anterior Eye. 2021;44(5

- Ruiz-Alcocer J, Martínez-Alberquilla I, Lorente-Velázquez A, Alfonso JF, Madrid-Costa D. Effect of defocus combined with rotation on the optical performance of trifocal toric IOLs. Eur J Ophthalmol. 2022;32(1):249-254. doi:10.1177/11206721211002121

- Ruiz-Alcocer J, Martínez-Alberquilla I, Rementería-Capelo LA, De Gracia P, Lorente-Velázquez A. Changes in Optical Quality Induced by Tilt and Decentration of a Trifocal IOL and a Novel Extended Depth of Focus IOL in Eyes With Corneal Myopic Ablations. J

Chapter 3:

- Crooke A, Martínez-Alberquilla I, García-Montero M, Rico-Del-Viejo L, Ruiz-Alcocer J, Madrid-Costa D. Impact of contact lens wear on NLRP3 gene expression: implications for ocular frailty in middle-aged adults. Exp Eye Res. 2021;202:108356. doi:10.1016/j.

- Martínez-Alberquilla I, Gasull X, Pérez-Luna P, Seco-Mera R, Ruiz-Alcocer J, Crooke A. Neutrophils and neutrophil extracellular trap components: emerging biomarkers and therapeutic targets for age-related eye diseases. Ageing Res Rev. 2022;74:101553.

- Crooke A, Martínez-Alberquilla I, Madrid-Costa D, Ruiz-Alcocer J. Presbyopia: an outstanding and global opportunity for early detection of pre-frailty and frailty states. Front Med. 2022;9:968262.

Communications

- Visual function, ocular surface integrity and symptomatology of a new extended depth-of-focus and a conventional multifocal contact lens. **Martínez-Alberquilla I**, García-Montero M, Ruiz-Alcocer J, Madrid-Costa D. 4th PhDay Complutense, Escuela de Doctorado UCM. Facultad de Óptica y Optometría. Online, October 15th 2020 (poster)

- Función visual, superficie ocular y sintomatología con una lente de contacto de profundidad de foco extendida y un diseño multifocal convencional. **Martínez-Alberquilla I**, García-

Montero M, Ruiz-Alcocer J, Madrid-Costa D. 26th Congreso Internacional de Optometría, Contactología y Óptica Oftálmica (OPTOM). Madrid, Spain, May 8th-28th 2021 (oral)

- Fonction visuelle, surface oculaire et symptomatologie d'une lentille avec profondeur de focus étendue et une lentille multifocale / [Visual function, ocular surface and symptomatology with an extended depth of focus contact lens and a conventional multifocal design]. **Martínez-Alberquilla I**, Lozano-Sierra I, Ruiz-Alcocer J, García-Montero M, Madrid-Costa D. 39th Congrès d'Optométrie et de Contactologie (COC2020). Paris, France. January 19th-20th 2020 (poster)

- Impact of contact lens wear on NLRP3 gene expression: implications for ocular frailty in middle-aged adults. **Martínez-Alberquilla I,** Crooke A, García-Montero M, Rico-Del-Viejo L, Ruiz-Alcocer J, Madrid-Costa D. 5th PhDay Complutense, Escuela de Doctorado UCM. Facultad de Óptica y Optometría. October 14th 2021 (poster)

- Impacto del uso prolongado de lentes de contacto en la expresión del gen NRLP3: implicaciones en la fragilidad ocular en adultos. **Martínez-Alberquilla I,** Crooke A, García-Montero M, Rico-Del-Viejo L, Ruiz-Alcocer J, Madrid-Costa D. Congreso Internacional de Optometría, Contactología y Óptica Oftálmica (OPTOM). Madrid, Spain, April 1st-3rd 2022 (oral)

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ANNEXES

Annex I. Ethics Committees Approval

Hospital Clínico San Carlos
SaludMadrid
Comunidad de Madrid

Informe Dictamen Protocolo Favorable
C.I. 19/007-R_P_BC
06 de febrero de 2019

CEIC Hospital Clínico San Carlos

Dra. Mar García Arenillas
Presidenta del CEIC Hospital Clínico San Carlos

CERTIFICA

Que el CEIC Hospital Clínico San Carlos en su reunión del día 16/01/2019, acta 1.1/19 ha evaluado la propuesta del promotor/investigador referida al estudio:

Título: Estudio de la calidad óptica, superficie ocular y sintomatología con diferentes tipos de lentes de contacto de hidrogel de silicona tras 15 días de uso.

Código Interno: 19/007-R_P_BC
Promotor: Facultad de Óptica y Optometría. Universidad Complutense de Madrid.
Versión Protocolo Evaluada: Protocolo 4CL
Versión Hoja Información al Paciente Evaluada:

Que en este estudio:

- Se cumplen los requisitos necesarios de idoneidad del protocolo en relación con los objetivos del estudio y están justificados los riesgos y molestias previsibles para el sujeto.
- Es adecuado el procedimiento para obtener el consentimiento informado.
- La capacidad del investigador y los medios disponibles son adecuados para llevar a cabo el estudio.
- El alcance de las compensaciones económicas previstas no interfiere con el respeto de los postulados éticos.
- Se cumplen los preceptos éticos formulados en la Declaración de Helsinki de la Asociación Médica mundial sobre principios éticos para las investigaciones médicas en seres humanos y en sus posteriores revisiones, así como aquellos exigidos por la normativa legal aplicable en función de las características del estudio.

Es por ello que el Comité **informa favorablemente** sobre la realización de dicho proyecto por el Dr. David Madrid Costa como investigador principal.

Lo que firmo en Madrid, a 06 de febrero de 2019

Hospital Clínico San Carlos
SaludMadrid

Dra. Mar García Arenillas
Presidenta del CEIC Hospital Clínico San Carlos

Hospital Clínico San Carlos
Profesor Martín Lagos, s/n. - Puerta G - 4ª Norte Madrid 28040 Madrid España
Tel. 91 330 34 13 Fax. 91 330 32 99 Correo electrónico ceic.hcsc@salud.madrid.org

Informe Dictamen Protocolo Favorable
C.P. Multi+Bio2019 - C.I. 19/028-R_P_BC
07 de febrero de 2019

CEIC Hospital Clínico San Carlos

Dra. Mar García Arenillas
Presidenta del CEIC Hospital Clínico San Carlos

CERTIFICA

Que el CEIC Hospital Clínico San Carlos en su reunión del día 16/01/2019, acta 1.1/19 ha evaluado la propuesta del promotor/investigador referida al estudio:

Título: Estudio de la función visual, superficie ocular, sintomatología y proceso de NETosis con dos tipos de lentes de contacto multifocales tras 15 días de uso en población présbita.

Código Interno: 19/028-R_P_BC
Versión Protocolo Evaluada:
Versión Hoja Información al Paciente Evaluada:

Que en este estudio:

- Se cumplen los requisitos necesarios de idoneidad del protocolo en relación con los objetivos del estudio y están justificados los riesgos y molestias previsibles para el sujeto.
- Es adecuado el procedimiento para obtener el consentimiento informado.
- La capacidad del investigador y los medios disponibles son adecuados para llevar a cabo el estudio.
- El alcance de las compensaciones económicas previstas no interfiere con el respeto de los postulados éticos.
- Se cumplen los preceptos éticos formulados en la Declaración de Helsinki de la Asociación Médica mundial sobre principios éticos para las investigaciones médicas en seres humanos y en sus posteriores revisiones, así como aquellos exigidos por la normativa legal aplicable en función de las características del estudio.

Es por ello que el Comité **informa favorablemente** sobre la realización de dicho proyecto por el Dr. David Madrid Acosta, como investigador principal en la Facultad de Óptica y Optometría de la UCM.

Lo que firmo en Madrid, a 07 de febrero de 2019

Hospital Clínico San Carlos
SaludMadrid

Dra. Mar García Arenillas
Presidenta del CEIC Hospital Clínico San Carlos

Annex II. Patient Information Sheet

"Estudio de la función visual, superficie ocular, sintomatología y proceso de NETosis con dos tipos de lentes de contacto multifocales tras 15 días de uso en población présbita"

Se le ha solicitado que participe en un estudio promovido por profesores investigadores. En este estudio se evaluará la función visual, superficie ocular, sintomatología y proceso de NETosis con dos tipos de lentes de contacto multifocales tras 15 días de uso.

¿Cuál es el fundamento y el objetivo de este estudio?

Los usuarios de lentes de contacto optan por este tipo de medida para corregir su graduación por diferentes motivos, y uno de ellos es la comodidad de no tener que llevar gafas. Las lentes de contacto se fabrican con diferentes materiales y diseños que buscan que la interacción de la lente de contacto en el ojo sea la menor posible, de forma que no produzcan visión borrosa durante su uso ni sean molestas.

Por tanto este estudio tiene como objetivo analizar el efecto de las LC, y de sus materiales, sobre los aspectos que afectan a una buena visión, confort e integridad y mantenimiento de unas estructuras oculares sanas: las células inmunes de la superficie ocular y los mecanismos moleculares que modulan dichas células pueden conducir a molestias e incluso enfermedades de la superficie ocular.

¿Es necesario que participe?

Su participación es absolutamente voluntaria. Si decide participar, deberá firmar un formulario de consentimiento. Aun así, podrá retirarse del estudio en cualquier momento sin dar ninguna explicación. Si decide abandonar el estudio, le rogaríamos que estableciera contacto con nosotros.

¿Cómo se efectuará el estudio?

Este estudio consiste en la realización de unas pruebas no invasivas, mientras usted usa las lentes de contacto que se le van a asignar previamente. El tiempo de estudio en cada visita será de una hora y media minutos.

Para analizar el efecto bioquímico-molecular de las lentes de contacto en paciente con presbicia, se tomarán muestras oculares en la pre-adaptación de las lentes de contacto y tras 15 días del uso de estas. Dichas muestras se obtendrán mediante dos técnicas: citología de impresión (para obtener células de la superficie ocular) y el test de Schirmer (para recoger lágrima), técnicas no invasivas que permitirán evaluar el efecto de las lentes de contacto sobre las células inmunes y sobre los mecanismos moleculares que modulan dichas células.

¿Cuáles son los posibles efectos secundarios, los riesgos y las molestias derivadas de mi participación?

El estudio no tiene como objetivo evaluar ni comparar la eficacia de ningún fármaco o tratamiento. No se trata pues, de un ensayo clínico, sino de un estudio de recogida de datos y muestras sistematizada, que permitirá analizar el efecto de las lentes de contacto sobre las células inmunes y los mecanismos moleculares que modulan y que están implicados en la aparición de molestias y/o enfermedades ocasionadas por el uso de estas. No ha sido reportado ningún daño derivado de los procedimientos diagnósticos que se le van a realizar, sin embargo, durante la recogida de muestras oculares puede sentir los siguientes síntomas:

-Leve incomodidad
-Enrojecimiento ocular leve

¿Se tratará de manera confidencial la información recogida en el estudio?

Durante el estudio se recogerán datos sobre su estado de salud, la fecha de nacimiento y los resultados de las exploraciones. Esta información se procesará y guardará en un fichero de investigación clínica de esta Universidad, con el único fin del estudio de los datos, que se tratarán de forma rigurosamente confidencial. El responsable del fichero será el investigador principal del estudio. El resultado del estudio se podrá publicar en una revista médica, pero sin desvelar su identidad. En todo caso se cumplirá atendiendo a la legislación de la Unión Europea en materia de protección de datos según el Reglamento General de Protección de Datos (General Data Protection Regulation, "GDPR") 2016/679 con entrada en vigor el 25 de Mayo de 2018, por lo que usted tiene derecho de acceso, rectificación, cancelación y oposición a los datos recogidos. Para poder ejercitar esos derechos debe dirigirse a Prof. David Madrid Costa. Facultad de Óptica y Optometría. Universidad Complutense de Madrid. Avda. Arcos de Jalón, 118. 28037 Madrid.

Los datos y muestras biológicas recogidas para el estudio estarán identificados mediante un código, de manera que no incluya información que pueda identificarle, y sólo el investigador principal del estudio/colaboradores podrá relacionar dichos datos/muestras con usted y con su historia clínica. Por lo tanto, su identidad no será revelada a persona alguna salvo excepciones en caso de urgencia médica o requerimiento legal.

El tratamiento, la comunicación y la cesión de los datos de carácter personal de todos los participantes se ajustarán a lo dispuesto en esta ley.

De acuerdo con lo que establece la legislación de protección de datos, usted puede ejercer los derechos de acceso, modificación, oposición y cancelación de datos, para lo cual deberá dirigirse al investigador principal del estudio. Si usted decide retirar el consentimiento para participar en este estudio, ningún dato nuevo será añadido a la base de datos, pero sí se utilizarán los que ya se hayan recogido.

En cuanto a las muestras biológicas recogidas en el estudio, sólo se utilizarán con fines de investigación por el investigador principal/colaboradores y no se harán nuevos estudios de investigación, con las muestras sobrantes, sin la aprobación previa del Comité Científico y del Comité de Ética de Investigación y Experimentación Animal.

Por último, es posible que los representantes designados por las autoridades sanitarias o el comité de ética que apruebe el estudio inspeccionen su historial para confirmar que el estudio se ha ejecutado de modo correcto. Toda esta información se tratará de manera rigurosamente confidencial.

Annex III. Informed consent

Título del estudio: ***"Estudio de la función visual, superficie ocular, sintomatología y proceso de NETosis con dos tipos de lentes de contacto multifocales tras 15 días de uso en población présbita"***

Investigador principal: _____

Nº de identificación del sujeto: _____

1. Confirmo que he leído y entendido la hoja de información para el paciente, que he tenido el tiempo suficiente para pensar acerca de mi participación en el estudio, que he tenido la posibilidad de hacer preguntas y que he obtenido una respuesta satisfactoria a mis preguntas. ☐

2. Entiendo que participo de manera voluntaria en este estudio y que soy libre de abandonarlo en cualquier comento sin que ello afecte a la calidad de atención que reciba fuera de este estudio o a mis derechos legales. ☐

3. Entiendo que las imágenes de mis ojos y las muestras de lágrima y células superficiales oculares serán recogidas de la manera descrita en la hoja de información para el paciente. ☐

4. Entiendo que las muestras biológicas solo serán usadas para los objetivos de este estudio. ☐

5. Entiendo que la información recogida relativa a mi persona puede ser consultada en el centro del estudio, el comité de ética y las autoridades regulatorias competentes para garantizar que el estudio se lleva a cabo adecuadamente. ☐

6. Entiendo que la información anónima acerca de mi persona se usara únicamente con los fines descritos en la hoja de información para el paciente. ☐

7. Entiendo que, de retirar mi consentimiento, todos los datos y las muestras recogidas previamente se conservarán y se usarán únicamente para los fines de estudio. ☐

8. Acepto participar en este estudio. ☐

Nombre del participante Fecha Firma

Nombre de la persona que recibe el consentimiento (si no se trata del IP) Fecha Firma

Annex IV. OSDI questionnaire

¿Ha experimentado alguno de los siguientes síntomas en la última semana?

	Constantemente	La mayor parte del tiempo	La mitad del tiempo	Ocasionalmente	Nunca
Ojos sensibles a la luz	(4)	(3)	(2)	(1)	(0)
Sensación de arena en los ojos	(4)	(3)	(2)	(1)	(0)
Dolor o irritación ocular	(4)	(3)	(2)	(1)	(0)
Visión borrosa	(4)	(3)	(2)	(1)	(0)
Visión reducida	(4)	(3)	(2)	(1)	(0)

¿Los problemas oculares le han dificultado alguna de las siguientes tareas en la última semana?

	Constantemente	La mayor parte del tiempo	La mitad del tiempo	Ocasionalmente	Nunca	N/S, N/C
Leer	(4)	(3)	(2)	(1)	(0)	(0)
Conducir de noche	(4)	(3)	(2)	(1)	(0)	(0)
Trabajar con ordenadores, usar cajero automático	(4)	(3)	(2)	(1)	(0)	(0)
Ver la televisión	(4)	(3)	(2)	(1)	(0)	(0)

¿Ha sentido molestias oculares en alguna de las siguientes situaciones en la última semana?

	Constantemente	La mayor parte del tiempo	La mitad del tiempo	Ocasionalmente	Nunca	N/S, N/C
Viento	(4)	(3)	(2)	(1)	(0)	(0)
Zonas de humedad baja (muy secas)	(4)	(3)	(2)	(1)	(0)	(0)
Sitios con aire acondicionado	(4)	(3)	(2)	(1)	(0)	(0)

Annex V. Clinical sheets

"Estudio de la repetibilidad de las medidas tomadas con el queratómetro K5M asociadas al uso de lentes de contacto"

FECHA:
ID PARTICIPANTE:

CONSENTIMIENTO INFORMADO: ☐

ANAMNESIS
Hª general: Hª ocular: Medicación: Usuario LC:

Examen ocular: ☐

	Baseline		
	Measure 1	Measure 2	Measure 3
TMH (mm)			
NIKBUT- first (sec)			
NIKBUT-average (sec)			

NLR			
TLR			
NBR			
TBR			

	Day 1- 8h			**Day 1- after removal**		
	Measure 1	Measure 2	Measure 3	Measure 1	Measure 2	Measure 3
TMH (mm)						
NIKBUT-first (sec)						
NIKBUT-average (sec)						
NLR						
TLR						
NBR						
TBR						

	Day 15- 8h			**Day 15- after removal**		
	Measure 1	Measure 2	Measure 3	Measure 1	Measure 2	Measure 3
TMH (mm)						
NIKBUT-first (sec)						
NIKBUT-average (sec)						
NLR						
TLR						
NBR						
TBR						

<u>"Estudio de la función visual, superficie ocular, sintomatología y proceso de NETosis con dos tipos de lentes de contacto multifocales tras 15 días de uso en población présbita"</u>

FECHA:
ID PARTICIPANTE:

CONSENTIMIENTO INFORMADO: ☐

DÍA 0

ANAMNESIS
Hª general: Hª ocular: Medicación: Usuario progresivos / LC (diseño):

Examen ocular: ☐

	Rx gafa	ADD	AV monocular (lejos)	AV binocular (lejos)	AV binocular (cerca)
OD					
OS					

Refracción subjetiva:

	Esf	Cil (eje)	AV monocular (lejos)	AV binocular (lejos)	ADD	AV binocular (cerca)
OD						
OS						

Dominancia:

Topografía:

	HVID	ECC	K1	AX	K2	AX
OD						
OS						

OSDI: ☐

Citología conjuntival: ☐

Schirmer: ☐

DÍA 1

LC	Sph	ADD	BC	DIAM
OD				
OS				

Modelo LC: Biofinity MF / EDOF

Baseline:

	Medida 1	Medida 2	Medida 3
TMH (mm)			
Global redness			
Bulbar temporal redness			
Bulbar nasal redness			
Limbal temporal redness			
Limbal nasal redness			
NIKBUT-first			
NIKBUT-avg			

Tinción corneal:

Tinción conjuntival:

DÍA 15

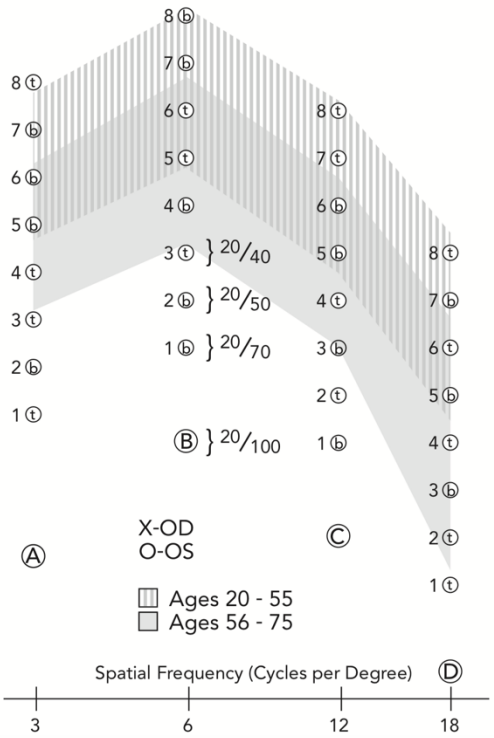
OSDI: ☐

Cuestionario halo y deslumbramiento: ☐

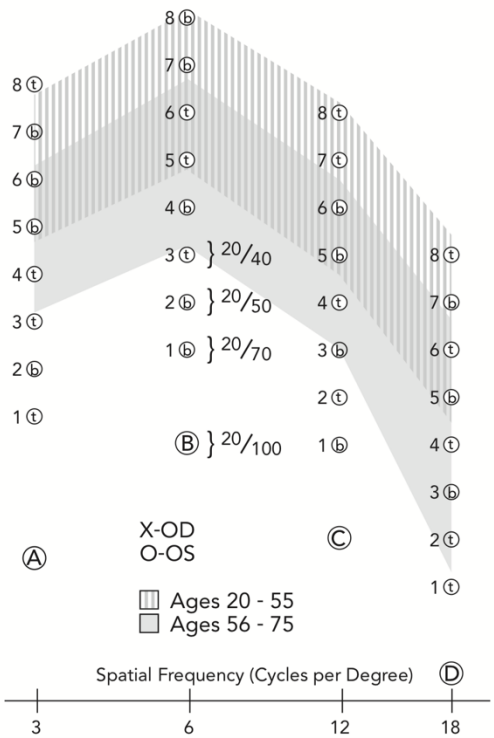
Curva de desenfoque (binocular and fotópica):

-3,50	-3,00	-2,50	-2,00	-1,50	-1,00	-0,50	0,00	+0,50	+1,00

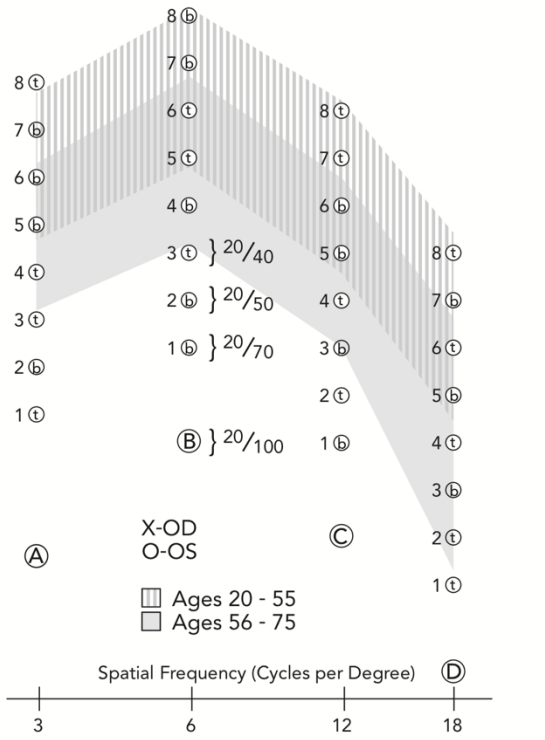
FOTÓPICO



MESÓPICO



MESÓPICO CON DESLUMBRAMIENTO



Tras retirada de LC:

	Medida 1	Medida 2	Medida 3
TMH (mm)			
Global redness			
Bulbar temporal redness			
Bulbar nasal redness			
Limbal temporal redness			
Limbal nasal redness			
NIKBUT-first			
NIKBUT-avg			

Tinción corneal:

Tinción conjuntival:

ARTICLE

Repeatability of Noninvasive Keratograph 5M Measurements Associated With Contact Lens Wear

María García-Montero, Ph.D., Laura Rico-del-Viejo, M.Sc., Amalia Lorente-Velázquez, Ph.D., Irene Martínez-Alberquilla, M.Sc., Jose Luis Hernández-Verdejo, Ph.D., and David Madrid-Costa, Ph.D.

Objective: To assess the intrarater repeatability of the measurements of tear meniscus height (TMH), noninvasive keratograph tear break-up time (NIKBUTs), and ocular redness measurements obtained with the Keratograph 5M (K5M) in a sample of soft silicone hydrogel contact lens (CL) wearers over 15 days.

Methods: Prospective study over two consecutive weeks. Three measurements of TMH, NIKBUTs (NIKBUT first and NIKBUT average), and ocular redness were obtained in different sessions; the first day (baseline, at 8 hr of wear, and after lens removal) and the last day of wear—15th day (at 8 hr of wear and after lens removal). The repeatability of measurements were assessed by two intraclass correlation coefficient (ICC) forms; single measurement [ICC (2,1)] and multiple measurements (k=3) [ICC (2, k)].

Results: Sixty-four eyes were analyzed. The repeatability of baseline TMH [ICC (2,1) greater than 0.90; coefficient of repeatability (CR)=0.06 mm] and after and during CL wear [ICC (2,k) greater than 0.90; CR≤0.07 mm] were excellent. The repeatability of baseline NIKBUT average [ICC (2,k)= 0.89 (0.82–0.93); CR=6.07 sec] was maintained after CL removal but was poorer during CL wear. The repeatability of baseline NIKBUT first [ICC (2,k) =0.80 (0.69–0.87); CR=8.74 sec] was maintained after CL removal and during CL wear at moderate–good level.

Conclusions: Intrarater repeatability of TMH, NIKBUTs, and ocular redness performed by K5M after CL wear remains stable when three measurements are performed. However, intrarater repeatability during CL wear decreased only for NIKBUT average and was not affected by time of use (15 days).

Key Words: Keratograph 5M—Repeatability—Contact lens—Tear film.

(*Eye & Contact Lens* 2019;45: 377–381)

Several biophysical tear film and ocular surface changes have been associated with contact lens (CL) wear.[1] Before fitting with CL, there are a number of tests available to the practitioner to assess the tear film and ocular surface to study the more convenient and more comfortable adaptation. The Oculus Keratograph 5M (K5M) (Oculus; Wetzlar, Germany) is a corneal topographer with multiple noninvasive features that analyzes the ocular surface, including tear meniscus height (TMH), noninvasive keratograph break-up time (NIKBUT) and bulbar and limbal redness assessment. It has been suggested that NIKBUT can be used as a tool to assess precorneal and prelens tear film quality for prescribing CL to study the tear film stability.[2] Likewise, it is important not to forget bulbar and limbal redness in CL wearers when redness is related to the extent of oxygen transmissibility of CL materials and may indicate corneal hypoxia.[3,4]

Therefore, clinical measures need to be repeatable, so that the quality of the clinical measures ensures a high-quality clinical service. However, one difficulty in the measurement error field is the number of different terms used to describe studies of measurement error in the medical literature. Reliability is the ability of scores of a measuring device to differentiate among subjects or objects, while repeatability is the degree of how close scores or ratings obtained under similar conditions are.[5]

The intrarater repeatability of K5M (Oculus) evaluation has been validated in previous studies in healthy eyes[6–9] and in dry eyes.[8,10] The repeatability of the parameters evaluated by the K5M is an essential requirement for use of the latter as a diagnostic tool or in the follow-up of interventions or ocular surface alterations (refractive surgery, dry eye). However, we have found no studies that validate intrarater repeatability in the management of CL patients. In addition, establishing the number of measures to be performed in a single assessment to ensure an acceptable level of repeatability of scores is another issue considered.[11]

Accordingly, the aim of current study was to assess the intrarater repeatability of measurements for TMH, NIKBUTs, and ocular redness in healthy subjects with soft silicone hydrogel (Si-Hy) CL wear.

MATERIAL AND METHODS

A prospective study was performed at the Faculty of Optics and Optometry of the Complutense University of Madrid. It was reviewed and approved by the Institutional Review Board of the Optometry Clinic, and all the procedures complied with the ethical principles of the Declaration of Helsinki. Informed consent was obtained from all patients once the purpose and possible consequences of the study had been explained to them. Inclusion criteria were to be current CL wearers who use CLs every day, 8 hr a day, in the age range from 18 to 55 years. Exclusion criteria included an active ocular allergy, refractive surgery, dry eye, or systemic medication known to affect tear film production. The

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evaluation performed on the first day, included review of medical history and anterior corneal surface evaluations.

The study was performed using a commercial monthly soft Si-Hy CL. All the participants underwent imaging with the Keratograph 5M (K5M; Oculus), an advanced corneal topographer with a built-in real keratometer and a color camera optimized for external imaging equipped with a modified tear film scanning function. Three measurements of the TMH, the first break of noninvasive keratograph tear break-up time (NIKBUT first), average break of noninvasive keratograph tear break-up time (NIKBUT average), and ocular redness were automatically obtained using Oculus K5M software according to the manufacturer's instructions. The TMH was measured perpendicular to the lid margin at the central point relative to the pupil center. Ocular redness scores were analyzed by R-scan software, and the automated redness scores evaluated were nasal limbal redness (NLR), temporal limbal redness (TLR), nasal bulbar redness (NBR), and temporal bulbar redness (TBR).

The study was conducted over 2 consecutive weeks. Previously, 1 week of washout without any CL was required to participate. Measurements were taken in different sessions on the first and last days of wear (15 days). On the first day of wear, measurements were taken before CL insertion (baseline), at 8 hr of wear, and after the lens was removed. On the last day of wear (after 15 days of use), measurements were taken at 8 hr of wear and after the lens was removed. Measurements without CL were registered 20 min after lens removal. The repeatability results were divided into 3 groups; baseline conditions, without the CL after 1 day and 15 days of use and during CL wear, at 8 hr of use on the first and 15th days.

Clinical examination was performed in the following order to minimize the effect of the assessment[12]: TMH, ocular redness, and NIKBUTs (NIKBUT first, NIKBUT average), and only the right eye of each participant was examined. The TMH score was obtained by the examiner from the registered image. NIKBUTs scores were obtained directly from the device. Ocular redness was obtained with the R-Scan software of the keratograph 5M. The R-Scan is a module that automatically and objectively classifies the bulbar and limbal degree of redness. The R-Scan detects the blood vessels in the conjunctiva and evaluates the degree of redness according the JENVIS grading scale.[13]

All the measurements were taken by the same examiner in a room with controlled temperature (24±1°C) and humidity (39%±2%). All subjects used the same CL solutions (Optifree Express Mds; Alcon Laboratories, Inc, Fort Worth, TX). Under repeatability conditions, independent test results were obtained using the same method, the same subject, the same examiner, and the same equipment with the shortest time possible between successive TMH and ocular redness readings. Noninvasive keratograph tear break-up time first and NIKBUT average readings were taken with an interval of at least 120 sec. Repeatability was assessed by performing three examinations in the various sessions.

The statistical analysis was performed using SPSS statistical package version 23 (SPSS, Inc, Chicago, IL). Normality for variables was checked using the Kolmogorov–Smirnov test. The intrarater repeatability for each parameter evaluated was assessed by means of repeated-measures analysis of variance and statistical parameters including the following statistical variables: within-subject SD (Sw), coefficient of repeatability (CR), within-subject coefficient of variation (CoV), intrasubject precision, intraclass correlation coefficient (ICC), and their 95% confidence intervals (CIs).

Two ICCs were calculated based on two ICC forms; two-way random effects, absolute agreement and single measurement [ICC (2,1)], and two-way random effects, absolute agreement and multiple measurements (k=3) [ICC (2, k)]. The ICC type (single measurement or average of measurements) provide results based on the number of measurements to be performed. The ICC (ranging from 0 to 1) assesses the consistency for data sets of repeated measurements within sessions.[14,15] The closer the ICC is to 1, the better the measurement consistency indicating that total variation in measurements is due solely to variability in the parameter being measured. The 95% CI of the ICC estimate (not the ICC estimate itself) should be used as the basis to evaluate the level of reliability. There are different scales to identify the value that indicates acceptable clinical reliability.[5,16] Intraclass correlation coefficient values below 0.5 can be indicative of poor reliability, values between 0.5 and 0.75 indicate moderate reliability, values between 0.75 and 0.9 indicate good reliability, and values above 0.90 indicate excellent reliability.[15,17]

TABLE 1. *Intrarater Repeatability of K5M Parameters Under Baseline Conditions Considering 2 Intraclass Correlation Coefficient Types: Single Measurement and Average of Measurements*

	Baseline														
			ICC Single Measure						ICC Average Measures						
	Mean	SD	ICC	95% CI for ICC	Sw	CoV (Sw)%	Precision	CR	ICC	95% CI for ICC	Sw	CoV (Sw)%	Precision	CR	Dif CoV (%)
TMH (mm)	0.28	0.01	0.95	0.92–0.96	0.02	8.03	0.04	0.06	0.98	0.97–0.99	0.01	4.74	0.03	0.04	3.29
NIKBUT first (sec)	10.56	7.02	0.57	0.43–0.69	4.61	43.66	9.03	12.77	0.80	0.69–0.87	3.15	29.87	6.18	8.74	13.78
NIKBUT average (sec)	12.74	6.54	0.73	0.61–0.82	3.43	26.94	6.73	9.51	0.89	0.82–0.93	2.19	17.20	4.29	6.07	9.74
NLR	0.73	0.42	0.87	0.81–0.91	0.15	20.47	0.29	0.42	0.95	0.93–0.97	0.09	12.39	0.18	0.25	8.08
TLR	0.46	0.27	0.75	0.65–0.83	0.14	30.17	0.27	0.38	0.90	0.85–0.94	0.09	19.07	0.17	0.24	11.10
NBR	1.12	0.44	0.89	0.84–0.93	0.15	13.01	0.29	0.41	0.96	0.94–0.97	0.09	7.82	0.17	0.24	5.19
TBR	0.68	0.28	0.78	0.69–0.85	0.13	19.66	0.26	0.37	0.91	0.87–0.94	0.08	12.30	0.16	0.23	7.36

95% CI, 95% confidence interval; CoV, coefficient of variation of Sw; CR, coefficient of repeatability; Dif CoV(%), differences between single-measure CoV and average-measure CoV; ICC, intraclass correlation coefficient; NBR, nasal bulbar redness; NIKBUT average, average break of noninvasive keratograph tear break-up time; NIKBUT first, first break of noninvasive keratograph tear break-up time; NLR, nasal limbal redness; Sw, within-subject SD; TBR, temporal bulbar redness; TLR, temporal limbal redness; TMH, tear meniscus height.

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The within-subject SD (Sw) is a simple way of estimating the SD of repeated measurements. The CoV was calculated as the ratio of the Sw to the overall mean and estimates the amount of variation seen between repeated measures taken from the same individual at different time-points. A smaller CoV means that the repeatability is higher. The intrasubject precision was defined as 1.96×Sw, which indicated the size of the range of error of the repeated measurements for 95% of observations. The CR was calculated using the within-session SD (S_w) of $1.96\times\sqrt{(2\times S_W^2)}$ or $2.77xS_w$,[15] which indicated the interval within which 95% of the differences between measurements are expected to lie.

RESULTS

Descriptive Statistics

Sixty-four eyes of 64 patients (48 men and 16 women; mean age 26.3±2.5 years; age range 21–32 years) were enrolled, and all the protocols in each session were followed. The baseline descriptive statistics for TMH, NIKBUT first, NIKBUT average, NLR, TLR, NBR, and TBR were 0.28±0.01 mm, 10.56±7.02 sec, 12.74±6.54 sec, 0.73±0.42, 0.46±0.27, 1.12±0.44, and 0.68±0.28, respectively. The descriptive statistics obtained in different sessions are summarized in Tables 2 and 3.

Intrarater Repeatability Under Baseline Conditions

The level of reliability considering ICC-type single measurement can be regarded as excellent for TMH measurements [ICC 0.95 (95% CI=0.92–0.96); CR=0.06], as good to excellent for NBR [ICC 0.89 (95% CI=0.84–0.93); CR=0.41] and NLR [ICC 0.87 (95% CI=0.81–0.91); CR=0.42], as moderate to good for TBR [ICC 0.78 (95% CI=0.69–0.85); CR=0.37], TLR [ICC 0.75 (95% CI=0.65–0.83); CR=0.38] and NIKBUT average [ICC 0.73 (95% CI=0.61–0.82); CR=9.51], and finally as poor to moderate for NIKBUT first [ICC 0.57 (95% CI=0.43–0.69); CR=12.77]. The level of reliability considering ICC-type average measurement for NIKBUT first can be regarded as moderate to good [ICC 0.80 (95% CI=0.69–0.87); CR=8.74]. Table 1 shows the results of repeatability analysis under baseline conditions.

Intrarater Repeatability After Contact Lens Removal

Table 2 shows the results of the reliability index measured with the K5M without the CL after 1 day and 15 days of use. The TMH repeatability was slightly poorer for ICC single values classified as good to excellent for both sessions: first and 15th day. However, the CR was 0.12 mm and 0.06 mm on day 1 and on day 15, respectively. Ocular redness repeatability on day 15 considering ICC-type average measurement can be regarded as excellent for NLR [ICC 0.97 (95% CI=0.95–0.98); CR=0.25], TLR [ICC 0.97 (95% CI=0.96–0.98); CR=0.15], NBR [ICC 0.96 (95% CI=0.94–0.98); CR=0.23], and TBR [ICC 0.97 (95% CI=0.95–0.98); CR=0.16]. The level of reliability considering ICC-type single measurement for NIKBUT first on day 1 and on day 15 was ICC 0.46 (95% CI=0.29–0.62); CR=12.25 and ICC 0.48 (95% CI=0.32–0.63); CR=12.75 respectively. Both of them can be regarded as poor to moderate.

Intrarater Repeatability During Contact Lens Wear

Table 3 shows the results of reliability scores measured during CL wear, at 8 hr of use on the first and 15th days. The level of reliability considering ICC-type average measurement can be

TABLE 2. *Intrarater Repeatability of K5M Parameters Measured Without the Contact Lens After 1 Day and 15 Days of Use Considering 2 Intraclass Correlation Coefficient Type: Single Measurement and Average of Measurements*

			ICC Single Measure						ICC Average Measures						
	Mean	SD	ICC	95% CI for ICC	Sw	CoV (Sw)%	Precision	CR	ICC	95% CI for ICC	Sw	CoV (Sw)%	Precision	CR	Dif CoV (%)
Day 1—after removing CL															
TMH (mm)	0.32	0.02	0.92	0.87–0.95	0.04	12.92	0.08	0.12	0.97	0.95–0.98	0.02	7.66	0.05	0.07	5.27
NIKBUT first (sec)	9.73	6.03	0.46	0.29–0.62	4.42	45.46	8.67	12.25	0.72	0.55–0.83	3.19	32.76	6.24	8.83	12.70
NIKBUT average (sec)	12.39	6.12	0.67	0.54–0.79	3.50	28.26	6.86	9.70	0.86	0.78–0.92	2.29	18.46	4.48	6.34	9.80
NLR	0.88	0.52	0.93	0.90–0.96	0.13	15.23	0.26	0.37	0.98	0.96–0.99	0.08	9.01	0.16	0.22	6.23
TLR	0.59	0.30	0.92	0.88–0.95	0.08	14.32	0.17	0.23	0.97	0.96–0.98	0.05	8.51	0.10	0.14	5.80
NBR	1.20	0.50	0.92	0.88–0.95	0.14	11.65	0.27	0.39	0.97	0.96–0.98	0.08	6.90	0.16	0.23	4.75
TBR	0.83	0.27	0.80	0.70–0.87	0.12	14.67	0.24	0.34	0.92	0.88–0.95	0.08	9.10	0.15	0.21	5.56
Day 15—after removing CL															
TMH (mm)	0.26	0.01	0.92	0.88–0.95	0.02	8.36	0.04	0.06	0.97	0.96–0.98	0.01	4.97	0.03	0.04	3.39
NIKBUT first (sec)	9.33	6.40	0.48	0.32–0.63	4.60	49.33	9.02	12.75	0.74	0.59–0.84	3.28	35.16	6.43	9.09	14.17
NIKBUT average (sec)	12.12	5.98	0.66	0.53–0.77	3.47	28.64	6.80	9.62	0.86	0.77–0.91	2.27	18.77	4.46	6.31	9.88
NLR	0.80	0.50	0.91	0.86–0.94	0.15	18.81	0.30	0.42	0.97	0.95–0.98	0.09	11.19	0.18	0.25	7.63
TLR	0.53	0.32	0.92	0.88–0.95	0.09	16.90	0.18	0.25	0.97	0.96–0.98	0.05	10.03	0.10	0.15	6.87
NBR	1.12	0.42	0.90	0.84–0.93	0.14	12.28	0.27	0.38	0.96	0.94–0.98	0.08	7.34	0.16	0.23	4.94
TBR	0.77	0.32	0.91	0.86–0.94	0.10	12.84	0.19	0.27	0.97	0.95–0.98	0.06	7.66	0.12	0.16	5.18

The impact on reliability index of contact lens wear.

95% CI, 95% confidence interval; CL, contact lens; CoV, coefficient of variation of Sw; CR, coefficient of repeatability; Dif CoV(%), differences between single measure CoV and average measure CoV; ICC, intraclass correlation coefficient; NBR, nasal bulbar redness; NIKBUT average, average break of noninvasive keratograph tear break-up time; NIKBUT first, first break of noninvasive keratograph tear break-up time; NLR, nasal limbal redness; Sw, within-subject SD; TBR, temporal bulbar redness; TLR, temporal limbal redness; TMH, tear meniscus height.

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regarded as excellent for TMH measurements on day 1 and day 15 {(ICC 0.97 [95% CI=0.95–0.98]; CR=0.05) and (ICC 0.96 [95% CI=0.95–0.98]; CR=0.04), respectively}. The level of reliability considering ICC-type average measurement can be regarded as moderate to good for NIKBUT first, on day 1, ICC 0.67 (95% CI=0.50–0.79); CR=10.74, and on day 15, [ICC 0.73 (95% CI=0.58–0.83); CR=9.31], and also for NIKBUT average, on day 15, ICC 0.70 (95% CI=0.54–0.82); CR=7.40. Regarding NIKBUT average, on day 1, the reliability index was ICC 0.63 (95% CI=0.43–0.77); CR=8.56 classified as poor to moderate.

DISCUSSION

Several studies have revealed the impact on ocular surface and tear-film stability of Si-Hy CL wear and have also reported changes in TMH,[18,19] NIKBUTs,[19–21] and bulbar redness.[19] Noninvasive or minimally invasive and objective tests have a major advantage over more invasive and subjective techniques to assess ocular surface parameters. Therefore, it is crucial to establish the degree of repeatability of the measures.

In the current study, the TMH, NIKBUTs, and ocular redness were measured using K5M in monthly Si-Hy CL-wearing patients. To the best of our knowledge, this is the first study to assess the repeatability of TMH, NIKBUTs, and ocular redness measured by K5M in monthly Si-Hy CL-wearing patients over 2 weeks.

Previous studies have reported the repeatability index of K5M parameters (TMH, NIKBUTs, and redness) in healthy eyes without CL. Tian et al.[8] studied the repeatability of TMH and NIKBUTs scores in healthy population (n=42). They reported ICC values of 0.84 (95% CI=0.75–0.90) and values of CR=0.14 mm for TMH scores. This ICC score can be classified as moderate to excellent. In reference to NIKBUTs scores, the limit of repeatability for NIKBUT first and NIKBUT average was 5.24 sec and 5.60 sec, respectively. Other authors found poorer repeatability limits for NIKBUTs scores; Markoulli et al.[7] concluded that the CR of NIKBUT first and NIKBUT average were 13.96 sec and 11.37 sec, respectively, and Fernandez et al.[6] found limits of agreement for NIKBUT first and NIKBUT average of 13.46 sec and 10.34 sec, respectively. Regarding ocular redness, Wu et al.[9] studied repeatability of bulbar redness in 30 eyes and observed values of ICC=0.947.

Our results under baseline conditions are in agreement with those previously reported, however, with some considerations. First, the TMH repeatability levels are better. Tear meniscus height measurement revealed an excellent level of repeatability and would enable a single TMH measurement in clinical protocols maintaining those levels. In this case, changes below 0.06 mm (CR=0.06) should not be considered clinical changes. Second, to maintain values of excellence (ICC>0.90) in terms of redness scores, three measurements need to be taken, with the NLR and NBR variables able to achieve those levels. And finally, the level of repeatability of NIKBUT average was classified as moderate to excellent with CR=6.07 sec based on 3 measurements. In case of only one measurement, the CR increased to values of 9.51 sec. Therefore, we recommend taking into consideration the ICC obtained, but giving special attention to the precision values and repeatability coefficients. Hence, if taking only one measure of NIKBUT average, it should be noted that changes of less than 9.51 sec cannot be justified as significant clinical changes. Regarding the NIKBUT first, it was the parameter with the poorest repeatability. We recommend performing at least 3 measurements, which ensures a moderate to good level with a CR of 13.78 sec and a CoV of 29.87%.

Despite the good reliability results in healthy eyes, some authors have studied reliability in dry eyes. Tian et al.[8] reported that, in dry

TABLE 3. *Intrarater Repeatability of K5M Parameters Measured During Contact Lens Wear, at 8 Hr of Use on the First and 15th Days, Considering 2 Intraclass Correlation Coefficient Type: single Measurement and Average of Measurements*

			ICC Single Measure						ICC Average Measures						
	Mean	SD	ICC	95% CI for ICC	Sw	CoV (Sw)%	Precision	CR	ICC	95% CI for ICC	Sw	CoV (Sw)%	Precision	CR	Dif CoV (%)
Day 1—8-hr wearing CL															
TMH (mm)	0.25	0.01	0.91	0.87–0.94	0.03	11.32	0.06	0.08	0.97	0.95–0.98	0.02	6.73	0.03	0.05	4.59
NIKBUT first (sec)	10.31	6.75	0.40	0.25–0.56	5.21	50.53	10.21	14.44	0.67	0.50–0.79	3.87	37.57	7.59	10.74	12.96
NIKBUT average (sec)	15.31	5.08	0.36	0.20–0.52	4.06	26.50	7.95	11.25	0.63	0.43–0.77	3.09	20.17	6.05	8.56	6.33
NLR	0.75	0.53	0.92	0.88–0.95	0.15	19.81	0.29	0.41	0.97	0.96–0.98	0.09	11.76	0.17	0.25	8.05
TLR	0.44	0.30	0.85	0.79–0.90	0.11	26.06	0.22	0.32	0.95	0.92–0.97	0.07	15.82	0.14	0.19	10.24
NBR	1.15	0.47	0.91	0.86–0.94	0.14	12.47	0.28	0.40	0.97	0.95–0.98	0.09	7.43	0.17	0.24	5.03
TBR	0.74	0.30	0.80	0.71–0.86	0.14	18.60	0.27	0.38	0.92	0.88–0.95	0.09	11.54	0.17	0.24	7.07
Day 15—8-hr wearing CL															
TMH (mm)	0.24	0.00	0.90	0.85–0.94	0.02	9.22	0.04	0.06	0.96	0.95–0.98	0.01	5.51	0.03	0.04	3.71
NIKBUT first (sec)	9.67	6.44	0.47	0.31–0.62	4.68	48.41	9.18	12.98	0.73	0.58–0.83	3.36	34.73	6.59	9.31	13.67
NIKBUT average (sec)	15.48	4.90	0.44	0.28–0.60	3.66	23.65	7.18	10.15	0.70	0.54–0.82	2.67	17.23	5.23	7.40	6.42
NLR	0.59	0.49	0.76	0.66–0.84	0.24	40.25	0.46	0.66	0.91	0.85–0.94	0.15	25.32	0.29	0.41	14.93
TLR	0.38	0.22	0.84	0.76–0.89	0.09	23.07	0.17	0.25	0.94	0.91–0.96	0.05	14.11	0.11	0.15	8.97
NBR	0.92	0.39	0.93	0.89–0.96	0.10	11.19	0.20	0.29	0.98	0.96–0.98	0.06	6.64	0.12	0.17	4.55
TBR	0.68	0.26	0.83	0.76–0.89	0.10	15.38	0.20	0.29	0.94	0.90–0.96	0.06	9.42	0.13	0.18	5.96

The impact on reliability index during contact lens wear.

95% CI, 95% confidence interval; CL, contact lens; CoV, coefficient of variation of Sw; CR, coefficient of repeatability; Dif CoV(%), differences between single measure CoV and average measure CoV; ICC, intraclass correlation coefficient; NBR, nasal bulbar redness; NIKBUT average, average break of noninvasive keratograph tear break-up time; NIKBUT first, first break of noninvasive keratograph tear break-up time; NLR, nasal limbal redness; Sw, within-subject SD; TBR, temporal bulbar redness; TLR, temporal limbal redness; TMH, tear meniscus height.

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eyes, the NIKBUTs were more reliable, but TMH was less reliable in patients with DED. On the other hand, Baek et al.[10] concluded that K5M was a useful noninvasive tool in the evaluation of the TMH in patients with dry eye syndrome, publishing CR values of 0.04 mm.

These results show that changes in the ocular surface can cause changes in the repeatability of the measurements. In addition, wearing CL implies an interaction between the lens and the ocular surface. Therefore, to validate intrarater repeatability of K5M in the management of CL patients is relevant. The current study addresses two aspects; first if CL wear can induce changes in the ocular surface that affect the levels of repeatability, and second, if CL and the time of use affect the repeatability index.

After CL removal (Table 2), the ICC average measure [ICC (2, k)] remained with the same rating (excellent, moderate, good, and poor) as under baseline conditions. In the case of TMH, three measurements should ideally be taken when this parameter is being evaluated after CL removal. This ensures that the levels of TMH repeatability will be the same as those obtained with a single measurement [ICC (2,1)] under baseline conditions. These results suggest that CL wear and time of wear does not induce changes in the analyzed variables that affect the K5M repeatability.

In terms of reliability index during CL wear (Table 3), our results suggest the advisability of performing three measurements while the TMH, NIKBUT first, and ocular redness kept the same [ICC (2, k)] rating. However, the repeatability of NIKBUT average decreased. The ICC average measure [ICC (2, k)] levels decreased in two grades, going from good–excellent under baseline conditions to poor–good during CL wear. Despite the loss of repeatability of the NIKBUT average, it has been observed that [ICC (2, k)] on the 15th day is better than on the first day. This indicates that CL can be an artifact for the NIKBUT average measurement during CL wear, while time of CL use is not an influencing factor (15 days). These results are particularly interesting because the NIKBUT average measurement is a major variable in the management of CL wear. With lens wear, the tear film undergoes extensive biophysical and biochemical changes, which have the potential to influence tear function and/or CL tolerance.[22] Therefore, the measurement, recording, and monitoring of ocular surface parameters are important in the management of CL wear.

In conclusion, intrarater repeatability of K5M for TMH, NIKBUTs and ocular redness after CL wear remained stable when three measurements were performed. However, intrarater repeatability during CL wear decreased only for NIKBUT average and was not affected by time of use (15 days).

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Visual function, ocular surface integrity and symptomatology of a new extended depth-of-focus and a conventional multifocal contact lens

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ABSTRACT

Purpose: To evaluate visual function, ocular surface integrity and dry eye symptoms with an extended depth-of-focus (EDOF) design and a conventional multifocal (MF) contact lens (CL) after 15 days of wear.
Methods: A crossover single mask randomised clinical trial was conducted including 30 presbyopes who used an EDOF and a conventional MF CL (Biofinity MF) for 15 days each. Defocus curves, depth-of-focus range, contrast sensitivity (CS) under photopic and mesopic conditions (with and without glare) and subjective perception of halos and glare were evaluated. The ocular surface was evaluated through non-invasive Keratograph tear breakup time (NIKBUT), averaged tear breakup time (NIKBUT-avg), tear meniscus height (TMH), bulbar and limbal redness, and conjunctival and corneal staining. Dry eye symptoms were assessed with the OSDI questionnaire.
Results: No statistically significant differences were found for defocus curves or depth-of-focus between the two CLs (both $p > 0.05$). Subjective perception of halos and glare was not significantly different between CLs. Statistically significant differences were observed for CS under mesopic conditions for low spatial frequencies ($p = 0.008$). None of the CL produced significant changes in NIKBUT, NIKBUT-avg, TMH or redness. No change in conjunctival staining was observed in 76.7 % and 73.3 % of participants for EDOF and Biofinity MF, respectively. No change in corneal staining was observed in 86.7 % and 83.3 % of participants for EDOF and Biofinity MF, respectively. No changes were observed in the symptomatology measured with OSDI questionnaire ($p > 0.05$).
Conclusions: Both CL for presbyopia offer good visual quality, preserve the ocular surface integrity and provide the patient with similar symptomatology levels after 15 days of lens wear.

1. Introduction

Over one billion people suffer from presbyopia [1]. Due to the increase in the presbyopic population associated with an ageing population, a rise in multifocal contact lens (CL) fitting will be expected in the next few years [2,3]. However, despite improvements in multifocal options and lens designs, it is estimated that approximately 63 % of presbyopes are fitted with non-presbyopic correction [2].

There are currently a wide variety of multifocal designs and it has been previously reported that the multifocality design directly affects the discontinuation rate [4]. Currently, simultaneous-image CLs are the most prescribed multifocal CLs [5]. However, these designs are often associated with dysphotopsias such as halos and glare perception, decreased contrast sensitivity [6] and ghosting [7]. Additionally, they are influenced by pupil variations and illumination levels [8,9], which might be detrimental for quality of vision.

To overcome these limitations, new designs for presbyopia are being developed varying high order spherical aberration terms to induce an extended depth-of-focus (EDOF) [10]. Based on theoretical modelling, these lenses are less susceptible to inherent ocular aberrations, pupil variations and CL decentration [11], which might alleviate visual disturbances associated with multifocal designs. EDOF provide significantly improved intermediate and near vision without compromising distance vision and are, theoretically, pupil-independent, which may significantly facilitate fitting process [10].

Additionally, an often hypothesised reason for the low multifocal CL

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Table 1
Inclusion criteria.

Inclusion criteria
Age between 45 and 65 years
Best corrected distance visual acuity 20/20 or better in both eyes
Near addition power ≥ 0.75 D
Astigmatism ≤ 0.75 D
No history of ocular surgery
No history of ocular or systemic disorder known to interfere with CL wear
Willing to provide informed consent and follow the protocol

Table 2
Contact lens parameters.

	EDOF	BIOFINITY MF
Company	Mark'ennovy	Cooper Vision
Material	Filcon V3	Comfilcon A
Water content (%)	75	48
Base curve (mm)	7.10 – 9.80 (step 0.30)	8.60
Diameter (mm)	13.50 – 15.50 (step 0.50)	14.00
Spheres (D)	+18.00 to −18.00 (step 0.25)	+6.00 to −6.00 (step 0.25) −6.50 to −10.00 (step 0.50)
Addition power (D)	0.75, 1.50, 2.25	1.00–2.50 (step 0.50)
Dk	60	128
Oxygen Transmissibility (Dk/t)	75 (ct −3.00 0.08)	142 (ct 0.09)
Modulus (MPa)	0.29	0.75

EDOF: extended depth-of-focus; Dk: oxygen permeability; ct: central thickness.

prescription rate is the absence of a multifocal CL providing adequate comfort and satisfying visual demands. In fact, presbyopes graded comfort and visual quality as equally important regarding CL dropout [12] and prefer CL correction when both factors are successfully achieved [13].

However, the great majority of studies carried out with CLs for presbyopia have mainly focused on visual performance. This means there is a need to design and develop more satisfactory options to correct presbyopia and consider both comfort and vision as crucial factors for CL fitting success.

Therefore, the aim of this study was to objectively evaluate visual function, ocular surface integrity and dry eye symptomatology with an EDOF CL design and a conventional aspheric multifocal CL throughout 15 days of wear.

2. Methods

2.1. Design

A prospective, crossover, randomised, single-masked (participant) clinical trial was conducted at the Complutense University of Madrid. The study conformed to the principles of the Declaration of Helsinki and obtained ethical approval from the Ethics Committee of San Carlos University Hospital (Madrid, Spain). Participant-masking was achieved by using non-labelled lens packaging. Examiner masking was not feasible due to differences in visibility tints and contact lens diameter.

2.2. Participants

A total of 30 presbyopic participants were enrolled in the study. Inclusion criteria are summarised in Table 1. The enrolled participants included existing CL wearers and non-CL wearers. Signed informed consent was obtained from all participants prior to study procedures. A one-week washout period was required for habitual CL wearers.

Table 3
Subjective perception of halos and glare. Questionnaire score description.

Frequency (0–3)	Intensity(1–3)	Bothersome(0–3)
0: Never		0: Not at all
1: Occasionally	1: Mild	1: A little
2: Quite often	2: Moderate	2: Quite
3: Very often	3: Severe	3: Very

2.3. Contact lenses

The study was carried out using an investigational prototype of EDOF CL (Mark'ennovy) and the commercially available Biofinity Multifocal (MF) CL (CooperVision). Both simultaneous vision CLs were fitted according to the manufacturer's fitting guide. The EDOF CL was customised, and its parameters were obtained from the manufacturer's automatic calculator based on keratometry readings, anterior corneal eccentricity and horizontal visible iris diameter, measured with the Keratograph 5 M (K5 M; Oculus GmbH). Lens details are shown in Table 2.

The EDOF prototype alters spherical aberration terms to provide a non-monotonic, non-aspheric, aperiodic, non-diffractive, refractive power profile across the optic zone [10]. This means there are distinct zones for distance and near powers changing abruptly and non-equally separated across the optic zone. On the other hand, the Biofinity MF uses an aspheric, monotonic and aperiodic design [14], which provides a gradual change in power distribution from distance to near power (centre-distance, CD) or vice versa (centre-near, CN).

Low (≤1.25D), medium (1.50−1.75D) and high (≥2.00D) addition powers were used for the EDOF lenses, based on the manufacturer's fitting guide. For the Biofinity MF, a CD design was fitted in the dominant eye, whereas a CN design was fitted in the non-dominant eye for addition powers higher than +1.50D. For additions of +1.50D or less, a CD design was fitted in both eyes. The dominant eye was determined using the +1.50D blur test [15]. Biotrue® Multi-Purpose solution (Bauch&Lomb, New York, USA) was used for disinfecting, cleaning and storing both lenses.

2.4. Clinical assessments

2.4.1. Visual performance

Binocular defocus curves were measured under photopic conditions (85 cd/m^2) over the range +1.00 to −3.50D in 0.50D steps with randomised letter sequences in order to reduce memory effects, Afterwards, the depth-of-focus range was defined as the range of focusing errors for which the visual acuity (VA) did not decrease below 0.1 logMAR. All the visual acuity measurements for the defocus curves were assessed using a distance visual acuity retro-illuminated ETDRS chart. Near visual acuity was checked with a near optotype but was not considered for the analysis.

Binocular contrast sensitivity (CS) function was measured using the CSV-1000 test (VectorVision, Greenville, Ohio) with a logarithmic scale for 3, 6, 12 and 18 cycles per degree (cpd) under photopic (85 cd/m^2) and mesopic (3 cd/m^2) illumination conditions. A glare source consisting of halogen lamps positioned in the middle of both vertical sides of the chart was used under mesopic conditions to measure CS function with the effect of glare. All CS measurements were taken at a recommended 2.5 m test distance. The results were expressed in logarithmic units for the statistical analysis.

Participants rated the frequency (0−3 scale) and intensity (1−3 scale) of halos and glare, and how bothersome (0−3 scale) these visual disturbances were. Questionnaire score description is explained in Table 3. A final score was calculated as follows: *Frequency x [(Intensity x Bothersome)/2]*, on a scale of 0–9.

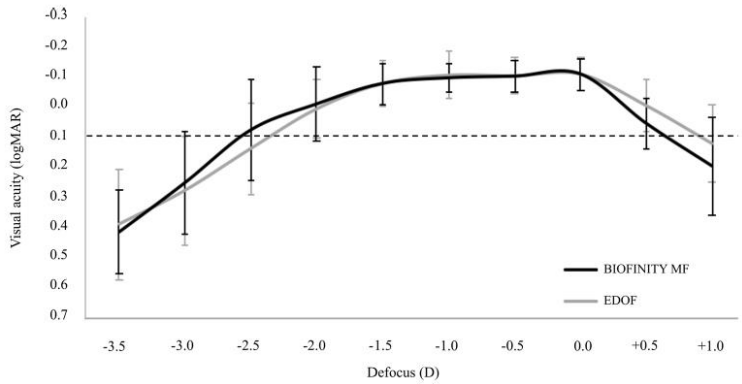


Fig. 1. Binocular defocus curves for EDOF CL and Biofinity MF on Day 15. Dash line indicates 0.1 logMAR visual acuity. Depth-of-focus range is defined as the range of vergences for which the visual acuity did not decrease below this line. $p > 0.05$ for all vergences. Data expressed as mean ± standard deviation.

2.4.2. Impact on ocular physiology

Ocular physiology was evaluated using the Keratograph 5 M. Measurements of tear meniscus height (TMH), objective non-invasive Keratograph first break-up time (NIKBUT), average of all break-up times (NIKBUT-avg), global ocular redness, bulbar temporal (BT), bulbar nasal (BN), limbal temporal (LT) and limbal nasal (LN) redness were obtained automatically from K5 M software. TMH values were measured perpendicularly to the lid margin in line with the pupil centre. For all redness parameters, the device generates a redness score based on the ratio between the blood vessels and the rest of the analysed area according to the JENVIS grading scale [16]. Corneal and conjunctival staining were measured using sodium fluorescein (FluoStrip, Contacare Ophthalmics and Diagnostics, Gujarat, India) and a cobalt blue filter. Images of staining were graded according to the Efron scale [17], range from 0 (normal) to 4 (severe).

2.4.3. Dry eye symptomatology

The Ocular Surface Disease Index (OSDI) questionnaire [18] was used to evaluate symptoms of ocular discomfort associated with dry eye. The questionnaire was completed under baseline conditions prior to lens fitting (after one-week washout period if necessary) and on Day 15 with each lens, and before conducting any clinical tests in order to avoid any influence on patient's response. The final score was calculated by the online calculator on a scale of 0–100 based on the following formula: OSDI = [(sum of scores for all questions answered) × 100] / [(total number of questions answered) × 4]. The higher the score, the more severe the symptoms and the greater the disability.

2.5. Study protocol

Visual performance metrics (defocus curves, contrast sensitivity and subjective perception of halos and glare) were measured on Day 15 of each lens wear (during lens wear). Ocular surface measurements were taken at baseline condition (with no lens and after one-week lens washout period) and on Day 15 of each lens right after CL removal. Participants were required to wear the lenses a minimum of 8 h per day. A one-week washout period was required between Day 15 of the first CL design and the baseline visit of the second CL.

All participants wore both CLs and the order of lens wear was randomised using a randomisation software (Randomisation generator, www.randomization.com). In order to minimise the effect of each clinical test on subsequent measurements, following the questionnaires, non-invasive tests were performed first, and corneal and conjunctival staining were performed last.

All examinations were performed by the same experienced CL practitioner. For the statistical analysis, one eye per participant was selected randomly.

2.6. Statistical analysis

The sample size was determined using the Epidat 4.2 software (http://dxsp.sergas.es) considering an error probability (α) of 0.05 and a required power (1-β) of 0.80, which produced an overall sample size of 20. 30 participants were recruited considering potential drop out during the study.

Descriptive and inferential data analyses were carried out using SPSS statistical package version 24 (SPSS, Inc, Chicago, IL). The Kolmogorov-Smirnov test was used to assess the normality of each variable. A two tailed paired *t*-test was applied for defocus curves and CS data to compare between Day 15 of each lens. The Wilcoxon signed rank test was applied for all other variables to compare between baseline and Day 15 of each lens (ocular surface variables and OSDI questionnaire), and between Day 15 of each lens (OSDI questionnaire and halo and glare questionnaire). Differences were considered statistically significant for $p < 0.05$.

3. Results

Thirty presbyopic participants (20 women and 10 men) with a mean age of 53.7 ± 5.3 years (range 45–64 years), with a mean myopic refraction of −2.18 ± 1.83 D, a mean hyperopic refraction of +1.84 ± 1.14 D (overall refractive error range from −5.50 to +4.00 D) and a mean near spectacle addition of +1.83 ± 0.40 D (range from +1.00 to +2.50 D) were enrolled in the study. Among the 30 participants, 20 were classified as high add, 8 as medium add and 2 as low add. 60 % of the participants had previously worn CLs, but none of them had worn multifocal CLs before.

3.1. Visual performance

Binocular defocus curves and depth-of-focus range values were measured on Day 15 after 8 h of wear. Fig. 1 shows no statistically significant differences for defocus curves between both CLs at any of the vergences (from −3.50 to +1.00 D) ($p > 0.05$ for all vergences). Depth-of-focus range values were 3.32 ± 0.61 D for EDOF CL and 3.21 ± 0.46 D for Biofinity MF, not being significantly different ($p = 0.521$).

Contrast sensitivity curves were measured on Day 15 after 8 h of wear. Fig. 2 shows binocular distance $\log_{10}$ CS under photopic, mesopic

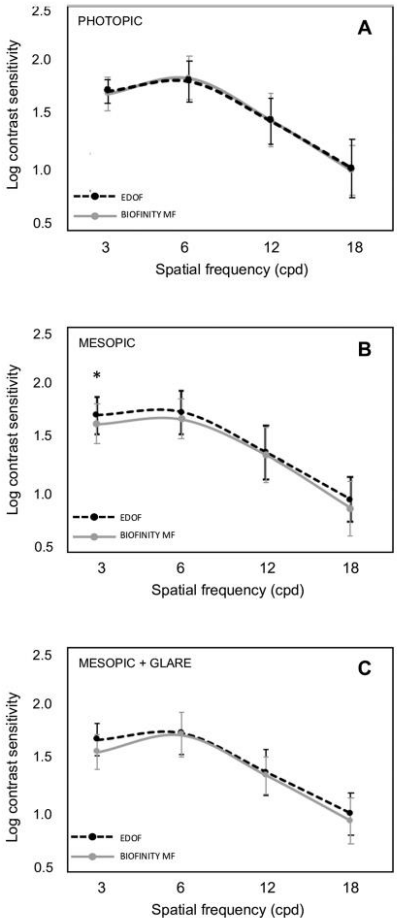


Fig. 2. Distance contrast sensitivity (CS) function on Day 15 with both multifocal CLs under photopic (A), mesopic (B) and mesopic with glare conditions (C). Data expressed in logarithmic units as mean ± standard deviation. Asterisk indicates statistical significance.

Table 4
Ocular surface parameters measured with the K5 M device. Results expressed as mean ± standard deviation.

	Baseline	Day 15	P-value
TMH (mm)			
EDOF	0.29 ± 0.11	0.28 ± 0.13	0.509
Biofinity MF	0.28 ± 0.10	0.27 ± 0.09	0.855
NIKBUT (sec)			
EDOF	9.31 ± 7.17	7.83 ± 5.75	0.648
Biofinity MF	8.32 ± 6.15	8.02 ± 5.87	0.776
NIKBUT-avg (sec)			
EDOF	12.36 ± 6.43	10.16 ± 5.86	0.191
Biofinity MF	11.34 ± 5.99	11.07 ± 6.26	0.650

EDOF: extended depth-of-focus; MF: multifocal; TMH: Tear meniscus Height; NIKBUT: Non-Invasive Keratograph Break-Up Time; NIKBUT-avg: average of all Break-Up Times.

and mesopic with glare conditions. Small statistically significant differences were observed for CS only under mesopic conditions for low spatial frequencies (3 cpd) ($p = 0.008$), being better for EDOF CL. The rest of the measurements remained similar between CLs ($p > 0.05$).

Both CLs induced a statistically similar perception of halos and glare visual phenomenon after 15 days of wear. Halo results were 1.36 ± 1.88 and 1.30 ± 1.42 units ($p = 0.687$) for EDOF and Biofinity MF, respectively, and glare results were 1.05 ± 1.29 and 1.20 ± 1.48 units ($p = 0.550$) for EDOF and Biofinity MF, respectively.

3.2. Ocular physiology

Regarding ocular surface parameters, Table 4 and Fig. 3 show the results of the variables measured with Keratograph 5 M (TMH, NIKBUT, NIKBUT-avg and ocular redness) at baseline and after 15 days of wear after CL removal. No statistically significant differences were found between both visits for any of the CL designs for TMH, NIKBUT, NIKBUT-avg and redness (all $p > 0.05$).

Changes in the conjunctival and corneal sodium fluorescein staining from baseline to Day 15 were evaluated for each lens. 76.7 % of participants exhibited no change in conjunctival staining after wearing the EDOF lens and 23.3 % increased by 1 point. For the Biofinity MF lens, 73.3 % of participants exhibited no change in conjunctival staining and 26.6 % increased by 1 point.

Regarding corneal staining, 86.7 % of participants exhibited no change after wearing the EDOF lens, while 13.3 % increased by 1 point. After wearing the Biofinity MF lens 83.3 % of participants exhibited no change while 16.6 % increased by 1 point.

3.3. Subjective ocular symptoms

Fig. 4 shows no statistically significant differences between baseline and after 15 days of lens wear for either design ($p = 0.073$ for EDOF, $p = 0.094$ for Biofinity MF) nor between Day 15 of each CL ($p = 0.110$).

4. Discussion

This crossover, randomised, single-masked clinical trial was performed to compared visual quality and performance, ocular physiological integrity and symptomatology of a novel extended depth-of-focus CL and a conventional aspheric multifocal design.

Defocus curves, depth-of-focus range, and contrast sensitivity function were measured to evaluate visual performance of two multifocal CL designs. Biofinity MF was considered as a representative of current multifocal CL designs, and was selected to participate in the study on account of a recent research [19], in which outperformed three other multifocal designs in terms of visual performance and impact on ocular physiology. Additionally, Biofinity MF is one of the most fitted multifocal contact lenses in the current market, which was an argument to choose these lenses. However, different contact lens design may have yielded different results.

The results of the current study supported that both simultaneous-image multifocal CLs provide good VA values for all vergences under photopic conditions, with no significant differences between designs. Biofinity MF obtained similar values in comparison to some studies [20] and slight variations compared to others [21], probably due to the difference in sample size. Additionally, defocus curve outcomes for EDOF were in accordance with a study conducted by Sha et al. [22] and slightly better compared to another study [23] conducted over 1 day, where multifocality adaptation played its role [20].

In regard to depth-of-focus range values, both multifocal designs performed similarly. Depth-of-focus, whether designed through manipulation of high order aberrations (EDOF) [10] or through the variation in the power profile across the lens (Biofinity MF) [24], provides comparable levels of depth-of-focus range between lenses.

To properly assess the visual function, CS was measured under

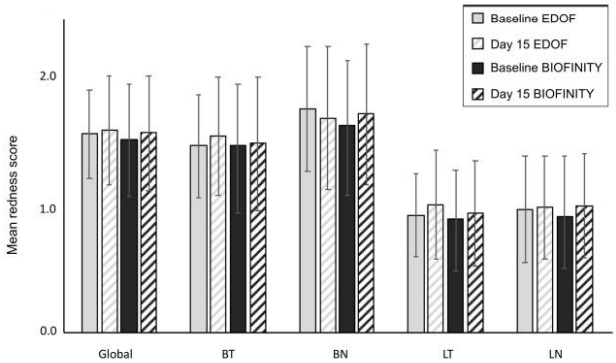


Fig. 3. Ocular redness measured with the K5 M device according to JENVIS grading scale for baseline and Day 15 of each CL. BN: bulbar temporal; BN: bulbar nasal; LT: limbal temporal; and LN: limbal nasal redness. Data expressed as mean ± standard deviation.

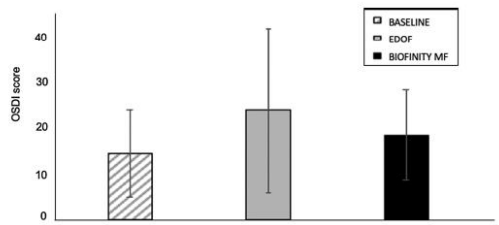


Fig. 4. OSDI values for baseline visit and after 15 days wearing EDOF and Biofinity MF CLs. Data expressed as mean ± standard deviation.

photopic, mesopic and mesopic with glare conditions. Previous studies have demonstrated that simultaneous-image multifocal CLs induce a deterioration of CS under photopic, mesopic and glare conditions when compared to monofocal or monovision [14,25]. Only statistically significant differences were found under mesopic conditions for low spatial frequencies, with EDOF obtaining better results. All other measurements were statistically similar between lenses, showing a comparable visual performance. CS function under photopic conditions for Biofinity MF is consistent with the literature for all spatial frequencies [21,26] and similar to another study [20] measured under glare conditions. However, these last results are not fully comparable because measurements with glare were taken under photopic conditions and not under mesopic conditions, as the current study.

EDOF results are also consistent with the literature for photopic CS for 18 cpd [23]. However, Bakaraju et al. [27] found slightly worse results for EDOF compared to the current study, mostly for 6 and 12 cpd under photopic conditions, probably due to multifocality adaptation (one more week of CL wear in the current study). Overall visual performance outcomes showed that EDOF provided comparable results to Biofinity MF with the added value of less pupil-dependence [11].

Beyond objective measurements, subjective measurements should be taken into consideration when it comes to evaluating patient satisfaction and visual performance with multifocal CLs [22,28]. Multifocality is known to induce deterioration in halometry [29]. However, no differences were found between CLs for subjective halo and glare perception, showing comparable results as well as being in accordance with a study conducted with EDOF CL and an aspheric design [22]. It should be noted that the questionnaire used was not validated, which can be considered as a potential limitation.

To the best of our knowledge, most multifocal CL studies focus on visual performance rather than on presbyopic ocular physiology. Accordingly, different ocular surface parameters were measured in this study to evaluate the interaction between the CLs and the ocular surface of presbyopes. Ocular physiology variables are in accordance with a study evaluating multifocal CL wear in presbyopes after 4 weeks, where no differences were found after lens removal for corneal staining, bulbar and limbal redness for Biofinity MF [19]. Overall ocular physiology outcomes showed no significant changes after each CL wear, as expected due to the same wearing modality and material similarities.

Regarding OSDI findings, no significant changes were observed between the baseline visit and 15 days after lens wear, despite the increase in the values after wearing time. This means that the severity of dry eye symptoms does not seem to be clearly affected by CL type. Several studies supported these results, showing no differences in OSDI values between three different silicone hydrogel CLs in first time CL users [30], and in ocular comfort in presbyopic patients after wearing two different multifocal CLs with different material [22,31]. However, it should be noted that multifocal CLs are manufactured in the same material as monofocal CLs. That is, the presbyopic population wear the same material as the young population, despite the increase in dry eye symptoms with age [32]. A recent study indicated that CLs do not behave the same way in the young population as in presbyopes, who reported more discomfort levels after 8 h than non-presbyopes [33]. The question arises whether a longer wearing period would have affected differently the ocular surface, which would allow researchers to determine if presbyopes require new or improved CL materials.

Although OSDI questionnaire is a validated and reliable questionnaire in assessing dry eye symptoms, different surveys, especially contact lens-specific surveys, may have yielded different results.

Thus, the conclusions of this study are that both CLs for presbyopia offer good visual quality and perform similarly in all visual performance variables, despite different optical designs. Additionally, they keep stable the ocular surface integrity as well as provide the patient with similar levels of symptomatology after 15 days of wear.

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Declaration of Competing Interest

The authors report no declarations of interest.

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Effect of defocus combined with rotation on the optical performance of trifocal toric IOLs

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Abstract

Purpose: To objectively analyze the optical quality of the FineVision Toric intraocular lens (IOL) with two cylinder powers when different combinations of rotations and residual refractive errors are induced.

Methods: This study assessed the FineVision Toric IOL with two different cylinder powers: 1.5 and 3.0 diopters (D). Three different rotation positions were considered: centered, 5° and 10° rotated. An optical bench (PMTF) was used for optical analysis. The optical quality of the IOLs was calculated by the modulation transfer function (MTF) at five different focal points (0.0, 0.25, 0.50, 0.75, and 1.00 D).

Results: The MTF averaged value of the reference situation was 38.58 and 37.74 for 1.5 and 3.0 D of cylinder, respectively. For the 1.5 D cylinder, the combination of 5° of rotation with a defocus of 0.25, 0.50, 0.75, and 1.0 D induced a decrease on the MTF of 12.39, 19.94, 23.43, 24.23 units, respectively. When induced rotation was 10°, the MTF decrease was 17.26, 23.40, 24.33, 24.48 units, respectively. For the 3.0 D cylinder, the combination of 5° with 0.25, 0.50, 0.75, and 1.0 D of defocus, induced a decrease on the MTF of 12.51, 18.97, 22.36, 22.48 units, respectively. When induced rotation was 10°, the MTF decrease was: 18.42, 21.57, 23.08, and 23.61 units, respectively.

Conclusion: For both FineVision Toric IOLs there is a certain optical tolerance to rotations up to 5° or residual refractive errors up to 0.25 D. Situations over these limits and their combination would affect the visual quality of patients implanted with these trifocal toric IOLs.

Keywords

IOLs, lens/cataract, optics/refraction/instruments, optics/refraction/instruments, optics/refraction/instruments, intraoperative complications of cataract surgery

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Introduction

Preoperative astigmatism is a key factor to be addressed by surgeons for successful cataract surgeries and intraocular lens (IOL) implantation. Some data estimates that around 50% of patients that underwent cataract surgery present preoperative astigmatism over 1.00 D.[1] Therefore, the global impact of preoperative astigmatism in the quality of vision represents a challenge that exceeds the classical cataract surgical burdens.

To compensate preoperative astigmatism, toric IOL implantation is the main method used among clinicians.[2] One of the most important factors for successful surgeries with toric IOLs implantation is the reaching of a proper alignment of the IOLs.[3] It is easy to find some degree of rotation after IOLs implantation but in general, toric platforms show high rates of stability[3,4] and good clinical results.[2]

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Multifocal toric IOLs also showed good clinical results and high rates of spectacle independence of astigmatic patients for both distance and near vision.[5–7] It has been reported that the impact of rotations in distance vision is greater for multifocal (bifocal) toric than for monofocal toric IOLs.[8] Currently, trifocal toric IOLs are also available for surgeons and the impact of potential rotations of these IOLs has been recently reported.[9]

Furthermore, slight over-refractions are common after cataract surgery. However, these over-refractions have relevant clinical implications as they can worsen the distance vision. Hence, the analysis of the combination of some degree of rotation and residual refractive error after trifocal toric IOLs implantation may offer a useful clinical approach to potential clinical scenarios after surgeries. This analysis should be done under the same conditions to eliminate inter-subject variability and physiologic variables that may confound measurements in vivo. For this purpose, an optical bench represents an objective and non-invasive method, as previously reported.[9]

Therefore, the current research aims to objectively analyze the optical quality of a trifocal toric IOL with different cylinder powers when determined amounts of rotation are combined with positive residual refractive errors.

Methods

Intraocular lens designs studied

The trifocal toric IOL analyzed was the FineVision Toric IOL (PhysIOL; Liège, Belgium). This is a double C-loop IOL with 5° angulation made of 26% hydrophilic acrylic material (hydroxyethylmethylmethacrylate-co-methylmethacrylate copolymer) with a refractive index of 1.46.[9] The lens incorporates both UV and blue-light filtration and has been used in a recent study.[9] As in the case of the non-toric version, trifocality is reached by combining two bifocal apodized diffractive patterns for far/near and far/intermediate vision respectively.[9–11] The lens presents a biconvex aspheric design with −0.11 µm of spherical aberration for a 6.0 mm of pupil size. The overall diameter of the lens is 11.40 mm and the optic zone is 6.00 mm. The optical power of the lens goes from +6.0 to +35.0 D in 0.50 D steps. The addition powers of the lens are +1.75 and +3.50 D for intermediate and near vision respectively. The power of the sphere was +20.0 D. Cylinder powers of 1.5 and 3.0 D were considered as a representation of low and moderate-high astigmatism, respectively.[12]

Optical bench

The image quality of each IOL was assessed with the PMTF optical bench (LAMBDA-X, Belgium) with the Software version: 1.13.6, used in similar previous investigations.[9,11,13,14] This device complies with International Standard Organization (ISO) 11979-2 and 11979-9 requirements. The optical bench allows optical quality measurements at different frequencies, focal planes, and pupil apertures.

At the same time, for toric IOLs analysis and in order to simulate the principal meridians of the cornea, the optical bench presents specific horizontal and vertical targets to align the IOLs under study.

Optical quality assessment

The modulation transfer function (MTF) analysis is considered as a standard method when it comes to analyzing the optical quality of IOLs as optical systems.[15–20] An optical system's MTF characterizes the image degradation caused by the optical system[17] and drop in the MTF results could lead to a decrease of the optical system's performance.[17] Previous authors have related MTF values to visual function parameters such us visual acuity.[21] Therefore, to achieve the main aim of the current study, the MTF analysis was performed.

Pupil size could affect the power distribution of the light through multifocal designs.[10] In order not to limit that distribution of light through the lens, an extreme small pupil aperture was avoided. Then, the selected pupil aperture for the current research was 4.5 mm.[9] As reported in previous studies,[9,11,13,14,20] the MTF value was considered as the "average modulation value" which is the averaged values across all frequencies within the 0.0–100.0 cycles/mm range. This value is proportional to the area under the MTF curve between 0.0 and 120.0 cycles/mm.[9,11,13,14,20] These range comprise from low to high spatial frequency values. Higher spatial frequencies are related to a larger drop in contrast due to an optical system. Then, the higher MTF value in the curve the better optical quality of the lens. At the same time, MTF curves at different focal points and for all different positions were performed.

Impact of defocus and rotations

In the current work, the IOLs studied were analyzed for a centered position and with 5° and 10° of rotation. In order to analyze the impact in distance vision of combining these rotations with some degree of residual defocus, the optical quality was also analyzed in each case for different positive foci (0.25, 0.50, 0.75, and 1.00 D) around the distance focal point (0.00 D). These positive foci are related to the distance focal points of myopic residual errors.

Following the methodology of a recent study,[9] measurements were taken with the IOL meridians aligned with the specific targets of the optical bench for a centered position to simulate the principal corneal meridians. For the analysis of the degradation of the optical quality, the IOLs were rotated by 5° and 10° versus the aligned position and the optical quality was assessed at the specific targets. As the targets and the principal meridians of the IOLs are always

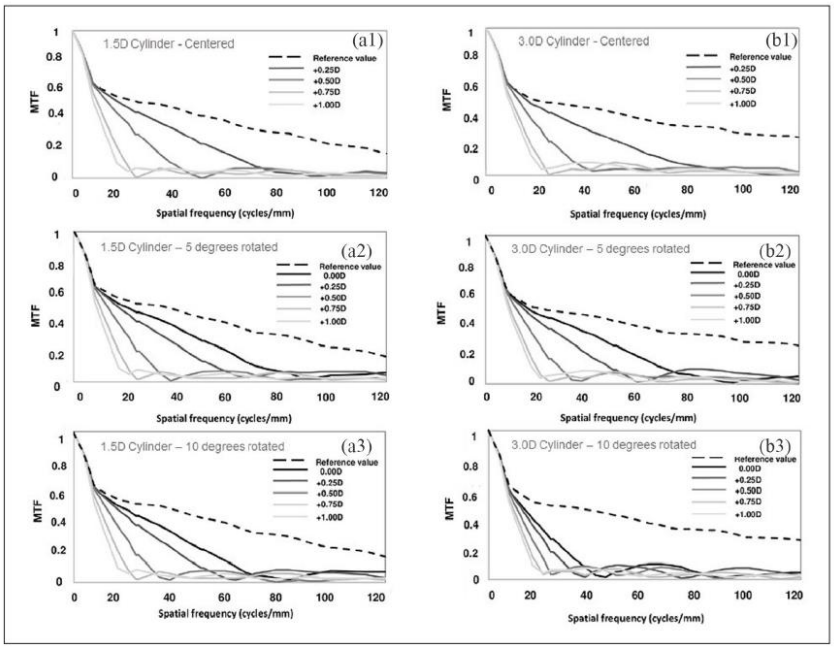


Figure 1. MTF curves at different object vergences (0.0, 0.25, 0.50, 0.75, and 1.0 D) for the low cylinder (a – left part of the figure) and the moderate-high cylinder lens (b – right part of the figure) at three different positions: centered (1), 5° rotated (2), and 10° rotated (3).

Table 1. Average modulation values of the lenses under study at different focal points (residual defocus) and for the three different positions (centered, 5° rotated, 10° rotated).

Vergence (D)	1.5 D cylinder			3.0 D cylinder		
	Centered (MTF values)	Rotated 5° (MTF values)	Rotated 10° (MTF values)	Centered (MTF values)	Rotated 5° (MTF values)	Rotated 10° (MTF values)
0.00	38.58*	28.89	27.02	37.74*	27.07	20.32
0.25	29.25	26.19	21.35	28.96	25.23	19.32
0.50	21.33	18.64	15.18	19.56	18.77	16.17
0.75	15.25	15.15	14.25	15.36	15.38	14.66
1.00	14.72	14.35	14.10	15.32	15.26	14.13

*Reference value.

perpendicular, the degradation of the image was assessed for only one (and always the same) target.

Previous investigations correlated MTF values with potential visual acuity changes. Felipe et al.[21] described that the visual acuity would vary by 0.18 decimal units per 10 units of average modulation variation under photopic conditions. Considering that the previously reported visual acuity at distance vision with this IOL was slightly over 20/20,[22] for the current study we considered 10 MTF units as the limit of tolerance which corresponds to almost two lines of vision.

Results

Figure 1 and Table 1 show the results of the IOLs with low cylinder power (1.5 D – left part of the figure) and moderate-high cylinder power (3.00 D – right part of the figure). The figure presents the MTF curves for all defocus

points (0.00, 0.25, 0.50, 0.75, and 1.00 D) for centered and rotated situations. Since the MTF for 0.00 D of defocus and for a centered position is expected to achieve the best optical results, this curve is presented as the reference value in all cases. On the other hand, the table compiles the average modulation values between 0.0 and 120.0 cycles/mm for all the specified situations. At the same time, the MTF averaged value for 0.00 D of defocus and for a centered position is showed as the reference value and all values are compared to this value. Regardless the amount of cylinder, the reference value showed to be similar.

Optical quality analysis: Rotation and defocus

The left-mid and left-bottom parts of the figure show results of the low cylinder (1.5 D) IOL with 5° and 10° rotation, respectively (A.2 and A.3). The right-mid and right-bottom parts of the figure show the outcomes of the moderate-high cylinder (3.0 D) IOL with 5° and 10° rotation, respectively (B.2 and B.3). For both cylinders, Table 1 shows that a defocus of 0.25 induced a decrease in MTF average value around 15 units. When the defocus is ⩾0.5 D the decrease was ⩾20 units. For 5° rotation, the results are similar to those of the centered position with 0.25 D of defocus. For this rotated situation, a defocus of 0.25 D did not induce a significant decrease of MTF average values (only 5 units vs rotated position without defocus and around 15 units vs the reference value). However, with a higher defocus the decrease was over 25 units.

A rotation of 10° was equivalent to a defocus of 0.50 D in a centered position, with a decrease in MTF average values over 20 units. Obviously, in this situation any combination with some amount of defocus also induced a decrease over 20 units versus the reference value.

Discussion

The current study analyzed the impact of different degrees of rotation combined with residual refractive errors in the optical quality of a trifocal toric IOL. A certain tolerance to low defocus errors and low degrees of rotations was found. Nevertheless, when these situations were combined the optical quality of the IOL decreased significantly.

Previous authors have reported clinical outcomes of the trifocal IOL under study.[22] However, to analyze the impact of the postoperative complications considered in this study, an optical bench was used. Beyond the differences between clinical and optical quality results, the latter could explain or justify different clinical outcomes[23,24] and could overcome the limits of classical clinical studies.

Both Figure 1 and Table 1 show the optical quality results for all the situations considered in the study. The first and the optimal situation to be analyzed for each IOL was the IOL without defocus (0.00 D object vergence) in a centered position, which was the "reference value". The MTF for the rest of the situations were compared to this reference in order to discuss the effect of defocus-rotation combinations.

As mentioned in the methods section, the visual acuity would vary by 0.18 decimal units per 10 units of average modulation variation under photopic conditions. Analyzing the results of both lenses (1.5 and 3.00 D of cylinder) (Figure 1; Table 1) in a centered situation (analyzing the table vertically), it is possible to observe that a defocus of 0.25 D induces a worsening under 10 MTF units versus each reference situation. This deterioration goes over 15 units for 0.50 D and over 20 units for 0.75 and 1.0 D of defocus. Hence, it could be considered a defocus of 0.25 D as tolerable. At the same time, in Table 1, it could be observed that a rotation of 5° (without defocus) induces a worsening comparable to 0.25 D of defocus. On the other hand, a rotation of 10° in the 1.5 D cylinder lens induces a worsening of 11.56 MTF units, being over the limit of tolerance. For the 3.0 D cylinder lens, 10° of rotation induces a decrease of 17.42 MTF units, which is significantly higher than the low cylinder case. For a combination of 0.25 D with 5° of rotation the worsening is similar for both lenses, being 12.39 and 12.51 units for the 1.5 and 3.0 D cylinder lenses, respectively.

Then these results suggest that there is certain tolerance to defocus up to 0.25 D or rotations up to 5°. However, a combination of 0.25 D and 5° exceed the limit of tolerance and could affect the visual quality. Obviously, for all potential combinations over 0.25 D and 5°, the optical quality of these trifocal toric IOLs decreased significantly (over 15 MTF units). In these cases, surgeons should consider surgical corrections for improving the visual quality of the patients.

The results showed in Table 1 present all potential combinations assessed in the study. These results, reading the table horizontally and/or vertically, could be a guide to understand whether reorientations or residual refractive correction (i.e. by corneal refractive surgery procedures)[25] could be the solution for restoring the visual quality of patients. For example, observing the results of the 3.00 D cylinder IOL at 0.25 D of defocus (horizontally), it is possible to observe that 10° of rotation induce a marked decrease in the optical quality. However, if the IOL is only reoriented (up to a centered position), the optical quality (and potentially the visual quality) could reach acceptable values. Another example could be the next defocus step, that is, the 3.00 D cylinder IOL at 0.50 D. Conversely, in the presence of a residual refractive error of 0.50 D, it is possible to observe that only a reorientation of the IOL itself would not achieve a tolerable value of visual quality. At the same time, only a surgical procedure for correcting this residual error could not provide a restoring of the visual acuity. Then, in that situation a reorientation should be combined with a correction of the residual refractive error.

Regarding the impact of residual refractive errors in the visual quality of patients implanted with trifocal IOLs, a previous investigation analyzed the effect of induced defocus with a trifocal (non-toric) IOL.[26] However, this previous study did not assess combinations of residual refractive errors and rotations (because it is impossible to analyze it in clinical studies). The results showed that defocus errors from 0.50 D affect the visual quality of patients at distance vision. Similarly, in our study, 0.50 D of defocus has a significant effect on the optical quality of the lenses and which could be correlated to those clinical results. This correlation confirm that our analysis could be a reliable approach to the visual quality of the patients. In addition, the analyzed combination of 0.25 D of defocus and 5° of rotation performed in our study has a similar impact if compared to 0.50 D of residual refractive error, confirming that the combination of low errors could induce a worsening of the visual quality.

We find it important to point out that the analysis through an optical bench does not fully represent a clinical scenario. But due to the aim of the study, this analysis is a useful and non-invasive methods for the simulated postoperative complications. In addition, some studies have reported a positive correlation of the results through optical simulation and their related clinical outcomes.[23] Despite the differences between clinical and theoretical approaches, the outcomes of the current study suggest that, even without defocus, rotations over 5° could affect the visual quality of the patients. Then, intraoperative position of trifocal toric IOLs seems crucial for clinical success with these premium lenses. Besides, the results as a function of defocus (residual refractive errors) show that these IOLs need for extreme accuracy in IOL calculation. This is due to two reasons: (1) Despite a certain tolerance, defocus errors of 0.50 D for centered IOLs show a decrease of the optical quality; (2) The results of the present study show that combinations of small residual refractive errors (0.25 D) with small amount of rotations (5°) are critical for the optical performance of these lenses.

To the best knowledge of the authors, this is the first work that objectively analyzed this trifocal toric IOL under the specified situations and no direct comparisons can be made with other studies. On the other hand, the lens under study is a widespread implanted IOL but other trifocal toric IOLs are commercially available. Therefore, these designs should be analyzed in similar future studies. Different pupil apertures and the analysis of residual refractive errors of different sign may give information of a wider postoperative refractive scenario. Finally, it would be interesting to analyze the impact of rotations and/or residual refractive errors at different distances of vision (multifocality) provided by these designs.

Despite the limitations of the study, the results could be the base for future studies that aimed to determine the limits of tolerance to misalignments and defocus errors with patients implanted with trifocal toric IOLs.

In conclusion, the results of the current study suggest that there is certain tolerance to defocus up to 0.25 D and rotations up to 5°. The combination of both situations (0.25 D and 5°) could decrease the optical quality beyond tolerable limits for the patients. For all situations and potential combinations over 0.25 D and 5°, the optical quality of these trifocal toric IOLs decreased significantly.

Declaration of conflicting interests

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ORIGINAL ARTICLE

Changes in Optical Quality Induced by Tilt and Decentration of a Trifocal IOL and a Novel Extended Depth of Focus IOL in Eyes With Corneal Myopic Ablations

Javier Ruiz-Alcocer, PhD; Irene Martínez-Alberquilla, MSc; Laureano A. Rementería-Capelo, MD; Pablo De Gracia, PhD; Amalia Lorente-Velázquez, PhD

ABSTRACT

PURPOSE: To assess the effect of decentration and tilt combined with prior myopic ablations on the optical performance of a trifocal intraocular lens (IOL) and a novel IOL with an extended depth of focus (EDOF) design.

METHODS: The XACT Mono-EDOF ME4 (Santen Pharmaceutical Co Ltd) and the trifocal FineVision (PhysIOL) IOLs were analyzed with and without simulated previous myopic ablations. The optical quality of the IOLs was evaluated with the PMTF optical bench (LAMBDA-X). The through-focus modulation transfer function (MTF) curves were recorded. Measurements were done for three situations: centered, 0.4 mm decentered, and 4 degrees tilted.

RESULTS: The trifocal IOL showed three peaks of vision and the EDOF IOL showed a far distance peak with intermediate addition. When decentration or tilt were induced, the trifocal IOL showed negligible changes but the EDOF IOL showed a -0.50 diopters (D) shift of the overall curve. With simulated myopic ablation, the trifocal IOL showed a -0.50 D shift of the curve. When tilt or decentration were also induced, the better optical results were found at -1.00 D. With myopic ablations, the EDOF IOL showed a -0.50 D shift of the optical quality and when decentration or tilt were then induced, negative shifts over -1.00 D were found.

CONCLUSIONS: The trifocal IOL was less affected by misalignments. When myopic ablations were induced, both lenses decreased their optical quality and the effects of misalignments were higher. In patients who have undergone corneal myopic ablation procedures, proper alignment of the implanted IOL and obtaining effective emmetropia becomes even more critical.

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Trifocal intraocular lenses (IOLs) are becoming more popular among surgeons who intend to provide spectacle independence.[1,2] Through the creation of three foci,[3] these designs have overcome the main limitation of bifocal IOLs, which is the gap between the optical quality peaks for near and far vision. As a result of this gap, the optical quality provided by bifocal IOLs for intermediate vision is low.[4,5]

Extended depth of focus (EDOF) IOL designs have also appeared on the market recently as an alternative to bifocal IOLs. The EDOF theoretical principle is to increase the depth of focus of the distance vision focal point by optimizing the interactions between higher order spherical aberration and diffractive patterns,[6-9] therefore reducing the gap between the peak performance for far and near. Despite a lower addition power, some studies have reported less photic phenomena when compared to classic diffractive or refractive bifocal and trifocal IOLs.[10,11]

Most current multifocal IOLs include aspheric designs attempting to neutralize the positive asphericity of the cornea. Conversely, myopic corneal refractive

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surgeries induce positive values of spherical aberration on the corneal surface.[12-14] Therefore, because a large number of patients have undergone myopic corneal refractive surgeries in the few past decades worldwide, it would be of great clinical interest to know how the asphericity of multifocal designs interacts with the described changes on corneal asphericity.

In addition, it is well known that visual axis misalignments after IOL implantation have an impact on the final visual quality of the patients.[15] It has also been shown that the eye's aberrations can interact with the optical design of the lens and produce both positive and negative synergies.[16,17] Therefore, the presence of tilt and decentration after IOL implantation is of utmost importance when evaluating the final optical quality provided by an IOL. The current study analyzes and compares the optical performance of both trifocal and novel EDOF IOL designs as a function of varying levels of tilt and decentration in eyes with a previous corneal myopic procedure. For the purpose of this study, we have chosen an optical bench to simulate the above-mentioned conditions with a non-invasive and reproducible technique.[18]

MATERIALS AND METHODS

IOLs Analyzed

The novel lens considered for the study was the hydrophilic XACT Mono-EDOF ME4 (Santen Pharmaceutical Co Ltd). This is a biconvex design with a C-loop platform. The lens shows diffractive rings in the anterior surface and a posterior asphericity designed to induce a negative Zernike 4th order spherical aberration coefficient of -0.17 µm for a 6-mm pupil. The diffractive rings are placed in the central 3 mm of the optical zone, diffracting light toward far and intermediate foci (from 1.00 to 1.90 diopters [D] of addition power). The overall diameter of the lens and optical zone are 12.5 and 6 mm, respectively. The base power of the EDOF lens evaluated in the current study was 18.50 D. At the same time, it incorporates an ultraviolet and blue-light blocker.[19]

The trifocal IOL analyzed was the hydrophilic version of the FineVision IOL (PhysIOL). This IOL presents an apodized diffractive trifocal design that combines two bifocal diffractive patterns for far/near and far/intermediate vision, respectively.[3,20,21,22] The IOL presents intermediate near additions of +1.75 and +3.50 D, respectively. The lens has a biconvex-aspheric optics with a posterior asphericity of -0.11 µm for a 6-mm pupil. The diameter of the lens is 10.75 mm and the optic zone's diameter is 6.15 mm. The base power of the trifocal lens analyzed in the study was also 18.50 D. The FineVision IOL incorporates an ultraviolet and blue-light blocker.

Optical Quality Analysis

The image quality of the IOLs was assessed with the PMTF optical bench (LAMBDA-X) with software version 1.13.6. The device complies with International Standard Organization 11979-2 and 11979-9 requirements and comes with additional lenses for an aberration-free model cornea. It allows modulation transfer function (MTF) measurements at various frequencies and at different focal planes (through-focus). The experimental set-up was assessed according to previous investigations.[3,23,24] Before each measurement, the optical device was calibrated to guarantee the precision of the MTF values.

To simulate post-myopic corneal procedures, the aberration-free cornea model was modified and an increment of +0.29 µm for 4.5 mm of pupil aperture was introduced in the optical system. This positive increment of spherical aberration intends to simulate low to moderate myopic ablations.[25] At the same time, both lenses with and without an increment of positive spherical aberration were analyzed for three positions: centered (reference measurement), with 0.4 mm of vertical decentration, and with 4 degrees of tilt.

Image Quality Metrics

Through-focus MTF curves comprising 11 different focal points (steps of 0.50 D) were calculated for a discrete spatial frequency of 50 cycles per millimeter.[3,23,24] This spatial frequency could approximately correspond to an optotype for 0.5 Snellen equivalent visual acuity in white light (30 cpd). The higher MTF value in the curve, the better optical quality the lens has at this focal point.

RESULTS

Figures 1-2 present the through-focus MTF of the EDOF IOL and the trifocal IOL, respectively. In both images, the blue line presents the curve for the centered situation, whereas the green line and the red line present the optical performance of the IOL through 0.4 mm of decentration and 4 degrees of tilt, respectively.

Figure 1A illustrates the through-focus MTF curves for the EDOF IOL itself and **Figure 1B** shows the through-focus MTF curves with the positive increment of spherical aberration that simulates corneas with prior low-moderate myopic treatments. For the EDOF lens itself, the curve shows two peaks at 0.00 D (far) and approximately -1.00 D/-1.50 D of object vergence (intermediate), with significant overlap between them. When decentration (green line) or tilt (red line) was induced, the far vision peak was shown to perform better for -0.50 D of vergence and both peaks showed less overlapping. When positive spherical aberration was induced,

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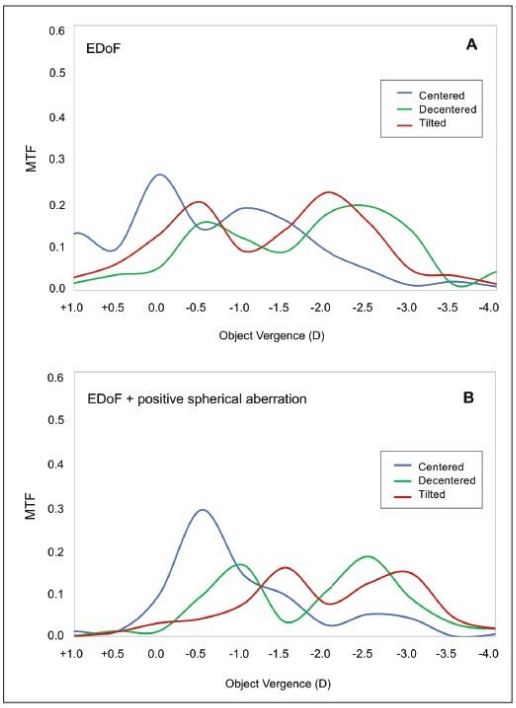


Figure 1. Through-focus modulation transfer function (MTF) curves for centered, decentered, and tilted situations of (A) the XACT Mono-EDOF ME4 intraocular lens (Santen Pharmaceutical Co Ltd) itself and (B) when a positive amount of spherical aberration was induced. The curves were calculated for a spatial frequency of 50 cycles per millimeter (cycles/mm). D = diopters

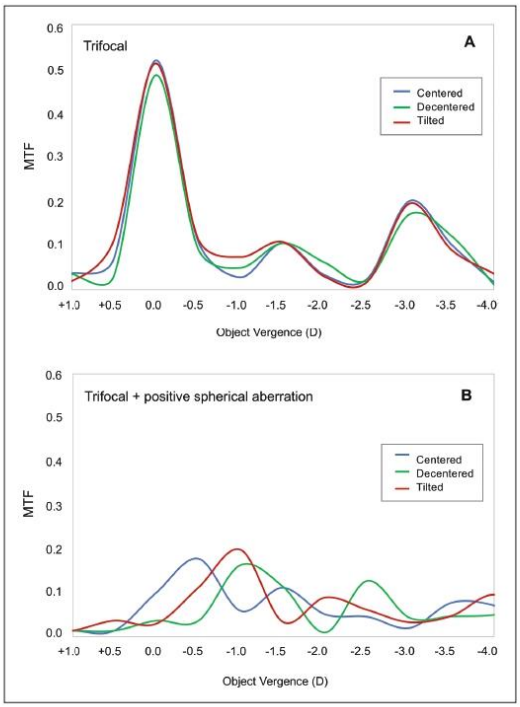


Figure 2. Through-focus modulation transfer function (MTF) curves for centered, decentered, and tilted situations of (A) the trifocal FineVision intraocular lens itself (PhysIOL) and (B) when a positive amount of spherical aberration was induced. The curves were calculated for a spatial frequency of 50 cycles per millimeter (cycles/mm). D = diopters

the centered EDOF lens showed a -0.50 D negative shift of the whole through-focus curve (blue line). For this centered situation, there was a drop of the intermediate peak and the overall curve showed only an elongated far distance peak. For the decentered situation, both far and intermediate peaks were differentiated, and the curve showed an overall negative shift of -1.00 D. For the tilted situation, both peaks were also differentiated, and the through-focus curve showed a significant negative shift of -1.50 D. In fact, the far distance peak was placed at the intermediate focal point and the intermediate peak was placed at a near focal point.

Figure 2A shows the results of the trifocal IOL and the through-focus curves show three differentiated peaks for far, intermediate, and near vision. When decentration (green line) or tilt (red line) was induced, the trifocal IOL showed similar results if compared to the centered position (blue line). Therefore, the trifocal IOL standing alone showed a noticeable tolerance to misalignments. **Figure 2B** illustrates the curves for the trifocal IOL with the positive increment of spherical aberration. In general, with the spherical aberration increment, the IOL maintained the three differentiated peaks of vision, but the optical quality of the far distance peak decreased if compared to the IOL standing alone. For the centered position (blue line), the spherical aberration increment induced a -0.50 D negative shift of the curve. When the lens was decentered (green line) and tilted (red line), the curves showed a negative shift of -1.00 D and both intermediate and near peaks were placed in nearer object vergences that correspond to shorter distances of vision.

DISCUSSION

Millions of patients have undergone corneal refractive surgeries in the past few decades and it is of inter-

est to analyze how these patients will perform with current multifocal designs. Considering that clinical studies are not able to analyze different situations in the same eye once the lens is implanted, in the current study we objectively analyzed the optical performance of a trifocal IOL and a novel EDOF IOL with an optical bench. For a more complete analysis, the lenses were analyzed standing alone and with a certain amount of positive spherical aberration that intended to simulate prior corneal myopic ablations. Potential misalignments after IOL implantation were analyzed and compared to a control situation (ie, for a visual axis centered position).

The results of the EDOF IOL were divided into the analysis of the lens itself and the lens with a certain amount of positive spherical aberration. The results of the lens itself in a centered position showed two overlapped peaks at distance and intermediate vision. The intermediate peak was placed between -1.00 and -1.50 D of object vergence. Beyond the intermediate peak, optical quality decreased steeply. As in previous studies that analyzed similar EDOF designs, this EDOF IOL actually had a bifocal design with an intermediate addition power.[26,27]

When the EDOF lens was decentered and tilted, it performed better at an object vergence of -0.50 D and both peaks were not overlapped, such as in the centered position. Despite this gap, the EDOF IOL still performs a theoretical addition of approximately 1.50 D. Then, when decentered or tilted, the EDOF IOL maintained an intermediate peak of vision but performed better at an object vergence of -0.50 D. This could decrease the optical quality at distance vision and provide an intermediate peak of vision, actually performing at nearer distances.

With a certain amount of spherical aberration, the centered EDOF IOL was shown to perform better at -0.50 D and maintained its optical quality for the far distance peak. However, there was a slight drop of the intermediate distance peak that reduced the addition to approximately 1.00 D. When decentration (green line) and tilt (red line) were induced, the shape of the curves changed but maintained far and intermediate peaks of vision. For the decentered situation, the peaks showed an overall negative shift of approximately -1.00 D. For the tilted situation, the through-focus curve showed an even higher negative shift. In fact, for this situation, the distance vision peak is placed at the intermediate object vergence and the intermediate vision peak is placed at the near object vergence. This situation would improve intermediate and near vision, but the degradation of the distance peak would be considerable.

The results of the EDOF IOL curves suggest that this IOL shows a certain tolerance to misalignments in terms of optical quality because they do not significantly affect the optical quality of far and intermediate peaks and the distances between them. However, the correct positioning of the lens is important because some degree of tilt and decentration may induce a negative shift in the overall through-focus curve of the lens. In addition, when a positive amount of spherical aberration is added to the IOL system, there is a myopic shift of the curve, but the lens maintains the optical quality. This suggests that for cases with a positive increment of spherical aberration (post-myopic ablations), a less positive IOL should be calculated to compensate for this myopization.[28,29] At the same time, if some amount of positive spherical aberration is induced and the lens is tilted or decentered, these misalignments induce a stronger negative shift that could decrease the visual quality of the patients significantly. Then, it is always important to achieve proper positioning of the IOL, but in cases of patients with higher amounts of positive spherical aberration, this alignment seems to be more important. Related to these results, previous studies have also reported that the combined cornea–IOL aberrations could reduce the optical properties of aspheric IOLs if the cornea–IOL system is misaligned[30] and that these misalignments could have a greater impact on eyes with previous myopic corneal refractive surgeries.[31]

Analyzing the through-focus curves of the trifocal IOL standing alone (without induced positive spherical aberration), it is possible to observe that the lens performs the classic three peaks of vision that correspond to the addition powers mentioned in the Methods section. These peaks have been reported in previous investigations and are related to good visual quality results at far, intermediate, and near distances.[32] The through-focus curves, when decentration and tilt are induced, show that the lens is highly robust to these misalignments, achieving similar results compared to the control situation and better results if compared to the EDOF IOL. The fact that the trifocal IOL is more robust to tilt and decentration than the EDOF IOL could be due to the trifocal IOL presenting a lower amount of negative spherical aberration. As was suggested in a previous study, this lower amount of negative spherical aberration would compensate for less positive corneal spherical aberration, but it could make the IOL more tolerant to misalignments.[33]

When a positive amount of spherical aberration was induced to simulate a previous myopic ablation (**Figure 2B**, blue line), the overall curve maintained the three peaks of vision but showed a myopic shift of -0.50 D. These results are similar to those obtained

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for the EDOF IOL and agree with other authors who analyzed patients with previous myopic corneal refractive surgeries who were implanted with multifocal IOLs.[28] These results suggest a less positive IOL calculation to compensate for the myopic shift induced under these conditions. In addition, the far distance peak was shown to be attenuated if compared to the situation without induced spherical aberration. On the other hand, when the lens was decentered and tilted, the through-focus curves also showed the three peaks but an overall negative shift of approximately -1.00 D. Therefore, despite the fact that the lens can offer three peaks of vision, if it is misaligned, the overall dioptric change suggests that a proper alignment is strongly recommended for patients with previous myopic corneal refractive surgeries. As in the case of the EDOF lens, the results also suggest that misaligned aspheric trifocal IOLs combined with significant increments of positive spherical aberration induce a noticeable worsening of the optical quality of the cornea–IOL system.

Then, in terms of optical quality tolerance, the EDOF lens was shown to be more robust to positive spherical aberration increments. However, if the lens is misaligned, it undergoes refractive changes (myopic shift) that are higher with previous corneal myopic procedures. Conversely, the trifocal IOL was shown to be robust to misalignments when the lens was analyzed standing alone and the optical quality at the far distance peak was shown to be significantly better if compared to the EDOF lens. Nevertheless, when a positive amount of spherical aberration is induced, the far distance peak decreased remarkably. As with the EDOF lens, the overall curve showed a myopic shift of -0.50 D. The actual clinical impact of a -0.50 D refractive shift should be determined in future studies, but it could be suggested that the higher refractive shift induced by misalignments would significantly impair the visual quality of patients implanted with these lenses. In general, the induced amounts of tilt and decentration showed a similar impact on the optical quality of both lenses (excepting the trifocal IOL standing alone). It would also be interesting to analyze whether a combination of both situations (decentration and tilt) may have a proportional impact (or not) on the optical quality of the lenses.

As far as the authors know, this is the first time that these situations were induced and analyzed by an objective method for these two multifocal IOLs. Therefore, future studies should address and correlate the clinical impact of the results obtained in this study. At the same time, it should be interesting to perform future similar studies with different amounts of spherical aberration to analyze these combinations and the impact on the optical quality of multifocal IOLs. With the same objective, simulating corneal ablations of different sign (ie, previous hyperopic corneal refractive surgeries) will also be valuable.

The optical quality provided by the trifocal IOL was more stable under the presence of decentration and tilt than that provided by the EDOF IOL. The changes in optical quality induced by the presence of tilt and decentration (for both lenses) were higher when a significant amount of positive spherical aberration was present. Surgeons should always aim for good visual axis centration of the IOL and to minimize lens tilt in all procedures but more even so in cases with patients who have undergone a previous corneal myopic procedure that wish to be implanted with a trifocal IOL design.

AUTHOR CONTRIBUTIONS

Study concept and design (JR-A, PD, AL-V); data collection (IM-A); analysis and interpretation of data (JR-A, LAR-C); writing the manuscript (JR-A, IM-A); critical revision of the manuscript (LAR-C, PD, AL-V); administrative, technical, or material support (AL-V); supervision (JR-A, PD)

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Impact of contact lens wear on NLRP3 gene expression: Implications for ocular frailty in middle-aged adults

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ABSTRACT

The inflammatory process plays a crucial role in frailty syndrome, which can appear in middle age and is associated with a poor health outcome. Consequently, gerontologists recommend screening inflammatory biomarkers in middle-aged adults to detect frailty and, therefore, prevent chronic diseases and mortality. External factors could be a risk factor for frailty because they can generate and extend the inflammatory process. For these reasons, we analysed the effect of long-term contact lens wear on mRNA level of genes linked to inflammation (IL-6, NLRP3, NK1R, CD73, MUC16 and TRPV1 genes) in conjunctival cells of middle-aged individuals, by quantitative PCR. Middle-aged contact lens wearers presented a significant increase of NLRP3 and MUC16 mRNA level as well as a decrease of CD73 mRNA level, in comparison with non-contact lens wearers. Additionally, we checked for a potential correlation between these transcript levels and clinical changes of the participants' ocular surface. Unlike molecular analysis, clinical examination fails to detect inflammation in contact lens wearers. These data suggest that long-term contact lens wear could trigger an inflammatory response in middle age orchestrated by NLRP3 inflammasome and modulated by CD73 and MUC16 proteins. Further studies are needed to confirm our gene expression findings at the protein level as well as to investigate the potential role of long-term CL wear in the onset of ocular frailty.

Middle age is a crucial period in life in which the decline of the body's regulatory systems (ageing) begins (Lachman et al., 2015; Lehallier et al., 2019). Indeed, Lehallier and colleagues have demonstrated a wave of proteomic change in the fourth decade of life that reflects the biological relevance of this period (Lehallier et al., 2019). Furthermore, various studies have detected frailty syndrome in middle-aged adults in whom this syndrome is a poor prognostic factor (Hanlon et al., 2018; Prince et al., 2019). Consequently, authors recommend screening frailty biomarkers in middle-aged people to prevent adverse health outcomes (Hanlon et al., 2018; Prince et al., 2019). The most convincing frailty biomarkers are inflammation-related candidates (Prince et al., 2019). Indeed, inflammation is a common pathway of frailty and age-related diseases (Fulop et al., 2010).

External factors could be a risk factor for frailty because they can generate and extend the inflammatory process. Indeed, they are pivotal in age-related ocular disease onsets (Crooke et al., 2017). Villani and colleagues have recently applied the concept of ocular surface frailty to an unhealthy ocular surface predisposed to age-related dry eye disease (Villani et al., 2019). In this context, contact lens (CL) wear could be a risk factor for this specific type of frailty. In relation to this issue, some authors have demonstrated that CL wear triggers an inflammatory response in young adults (Lopez-de la Rosa et al., 2019; Saliman et al., 2020). Moreover, CL wear is considered a risk factor of dry eye disease (Kojima, 2018). For these reasons, we have investigated the potential inflammatory effect of long-term CL wear in middle age.

Twenty-six middle-aged individuals (age range: 42–65 years), 12 non-contact lens wearers (NCLW; n = 12; mean [SD] age: 53.8 [7.0] years; 8 females and 4 males) and 14 contact lens wearers (CLW; n = 14; mean [SD] age: 53.9 [4.1] years; 9 females and 5 males) with a CL wearing routine of 8 h per day for at least 1 year were enrolled for this study. Individuals with active ocular allergy, ocular inflammatory diseases, refractive surgery or systemic medication were excluded. The study was approved by the ethics committee of the Hospital Clínico San Carlos and written informed consent was obtained from each

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participant.

All subjects underwent a standard clinical examination of the ocular surface (Wolffsohn et al., 2017) to evaluate the gross impact of CL wear. The clinical protocol included evaluation of ocular irritation symptoms and assessment of the tear film stability and the ocular surface health status.

The Ocular Surface Disease Index (OSDI) questionnaire (Schiffman et al., 2000)was used to evaluate symptoms of ocular irritation. The formula used by Schiffman and colleagues (Schiffman et al., 2000) was applied to obtain the final OSDI score. The higher the score, the more severe the symptoms and the greater the disability.

The tear meniscus height (TMH) and first/average non-invasive tear film breakup time (NIKBUT first/avg) parameters were determined to evaluate tear film stability. The bulbar redness (BR) and corneal/conjunctival integrity were determined to check the ocular surface health status.

The measure of TMH, NIKBUT first/avg and BR parameters were performed with the Keratograph 5M (Oculus GmbH, Wetzlar, Germany) device equipped with the modified tear film scanning function and obtained automatically from the K5M software following the manufacturer's directions. An assessment of corneal/conjunctival integrity was performed, 2 min after application of fluorescein sodium ophthalmic strips (FluoStrip, Contacare Ophthalmics and Diagnostics, Gujarat, India), with the Keratograph 5M (Oculus GmbH) device and the K5M blue-free barrier filter. Fluorescein corneal and conjunctival staining images were graded according to the Efron scale (Efron, 1998), ranging from 0 (normal) to 4 (severe). Conjunctival staining was divided into bulbar and tarsal sections.

After the clinical examination, and in order to evaluate the molecular impact of CL wear, a conjunctival specimen was obtained from each individual by impression cytology with the EYEPRIM device (OPIA Technologies, Paris, France). Conjunctival specimens were preserved in RNAlater solution (Thermo Fisher Scientific) overnight and, after removing the RNAlater, stored at – 80 °C until total RNA isolation.

Total RNA was isolated from impression cytology specimens using RNeasy Mini Kit (Qiagen, Madrid, Spain), following the manufacturer's instructions. First-strand cDNA synthesis was performed from 22 μl of total RNA, using High Capacity cDNA Reverse Transcription Kit and random hexamer primers (Thermo Fisher Scientific, Madrid, Spain). Quantitative PCR (qPCR) was performed in technical triplicates using cDNA, the Quantitect SYBR Green Kit (Qiagen) and gene-specific PCR primers for HPRT1 (QuantiTect Primer Assay QT00059066, Qiagen), IL-6 (QuantiTect Primer Assay QT00083720, Qiagen), NLRP3 (QuantiTect Primer Assay QT00029771, Qiagen), NK1R (5′-CTACTACTCAACCA-CAGAGAC-3′/5′-CACAGATGTGGTACACTTTC-3′, Merck, Madrid, Spain), CD73 (QuantiTect Primer Assay QT02451512, Qiagen), MUC16 (QuantiTect Primer Assay QT01192996, Qiagen) and TRPV1 (QuantiTect Primer Assay QT00046109, Qiagen), on an ABI Prism 7300 PCR System (Thermo Fisher Scientific). The thermal cycler program was 15 min at 95 °C, followed by 40 cycles of 15 s at 94 °C, 30 s at 55 °C and 34 s at 72 °C (data collection step). The HPRT1 gene was used as an internal control to normalise mRNA relative expression, after its validation for qPCR. Validation of the internal control gene and qPCR data analysis were performed by the $2^{-\Delta Ct}$ method (Schmittgen and Livak, 2008).

Statistical analysis was performed using IBM SPSS Statistics software version 25.0. (SPSS Inc., Chicago, IL, USA). Shapiro-Wilk and Mann-Whitney tests were used for statistical analysis of quantitative clinical (OSDI, TMH, NIKBUT first, NIKBUT avg and BR) and molecular (gene expression data of HPRT1, IL-6, NLRP3, NK1R, CD73, MUC16 and TRPV1) data. The chi-square test was used for statistical analysis of qualitative clinical data (corneal and conjunctival staining). All the values (clinical and molecular) are expressed as mean ± SD and median. A statistical significance of 95% was established ($p < 0.05$) in all the tests. Correlations among molecular and clinical variables were assessed through Kendall's Tau-b (τb) coefficient. The correlations were considered significant if $p < 0.05$.

To examine the mRNA level of inflammatory genes in conjunctival cells of NCLW and CLW by qPCR, we chose the HPRT1 gene as an internal control because its expression was unaffected by CL wear ($p = 0.43$) (Table 1). The levels of IL6, NK1R and TRPV1 mRNAs were comparable between CLW and NCLW ($p > 0.05$) (Table 1). Conversely, CLW showed a marked overexpression of NLRP3 and MUC16 genes (median 2.6-fold and 3.5-fold higher, respectively, than NCLW; $p < 0.05$) (Fig. 1A–B; Table 1). Likewise, we observed a significant down-regulation of CD73 gene in CLW (median 6.4-fold lower than NCLW; $p < 0.05$) (Fig. 1C; Table 1).

Concurrently, we checked if CL wear alters the ocular surface of middle-aged adults at the clinical level (Table 1). Clinical parameters levels were comparable between CLW and NCLW ($p > 0.05$) (Table 1).

Finally, we also studied the possible correlation between NLRP3, MUC16 and CD73 mRNA level and clinical parameters. We found only a significant negative correlation between tarsal conjunctiva staining and MUC16 mRNA level ($\tau b = -0.62$; $p = 0.04$).

This study shows, for the first time, the effect of CL wear on conjunctival NLRP3 and CD73 gene expression. Our results demonstrate a higher expression of the NLRP3 gene in CLW than NCLW. The NLRP3 inflammasome is a major contributor to the chronic low-grade inflammation that characterises frailty and age-related conditions including eye diseases (Fulop et al., 2010; Lim et al., 2020). Indeed, Niu and colleagues have proved the upregulation of NLRP3 mRNA and protein in ocular surface cells of dry eye patients (Niu et al., 2015). Furthermore, other authors have demonstrated that frail middle-aged adults present a race-specific alteration of IL1-β mRNA (an effector of NLRP3 inflammasome) in blood cells (Prince et al., 2019).

Additionally, we have demonstrated that CLW present a lower CD73 mRNA level than NCLW. CD73 is an ectoenzyme that, along with the CD39 nucleotidase, degrades the extracellular inflammatory nucleotide adenosine triphosphate (ATP) to adenosine, a potent endogenous anti-inflammatory agent (Hasko and Cronstein, 2013). In fact, ATP is a co-activator of NLRP3 inflammasome (Stagg and Smyth, 2010). Therefore, this result suggests that long-term CLW could be more vulnerable to the inflammatory process, due to the imbalance between the levels of

Table 1
Molecular and clinical parameters of the study groups.

	NCLW	CLW	p-value
Molecular parameters			
HPRT1	1.09 ± 0.46	1.10 ± 0.94	0.43
IL6	0.07 ± 0.10	0.11 ± 0.16	0.35
NLRP3	0.01 ± 0.01	0.05 ± 0.08	**0.02***
NK1R	0.00 ± 0.00	0.00 ± 0.01	0.46
CD73	1.72 ± 0.85	0.86 ± 1.08	**0.02***
MUC16	0.50 ± 0.28	1.78 ± 1.52	**0.04***
TRPV1	0.24 ± 0.08	0.29 ± 0.20	0.71
Clinical parameters			
TMH (mm)	0.30 ± 0.16	0.31 ± 0.07	0.50
BR	1.40 ± 0.36	1.33 ± 0.30	0.50
NIKBUT first (sec)	10.63 ± 6.86	10.01 ± 5.59	0.98
NIKBUT avg (sec)	13.73 ± 6.48	12.98 ± 5.08	0.65
OSDI	15.94 ± 9.06	13.19 ± 9.70	0.46
Corneal staining (Efron scale)	0.27 ± 0.65	0.00 ± 0.00	0.20
SBC staining (Efron scale)	0.00 ± 0.00	0.08 ± 0.28	1.00
IBC staining (Efron scale)	0.09 ± 0.30	0.08 ± 0.28	1.00
NBC staining (Efron scale)	0.73 ± 0.79	0.69 ± 0.75	1.00
TBC staining (Efron scale)	0.45 ± 0.52	0.23 ± 0.60	0.06
TC staining (Efron scale)	0.18 ± 0.40	0.15 ± 0.38	1.00

Note: Molecular data are the mean ± standard deviations (SD) of mRNA fold change. Clinical data are presented as the mean ± SD. * indicates statistically significant differences ($p < 0.05$) between NCLW and CLW. NCLW: non-contact lens wearers; CLW: contact lens wearers; TMH: tear meniscus height; BR: bulbar redness; NIKBUT first: non-invasive first tear film breakup time; NIKBUT avg: average time of all tear film break-up incidents; OSDI: Ocular Surface Disease Index questionnaire; SBC: superior bulbar conjunctiva; IBC: inferior bulbar conjunctiva; NBC: nasal bulbar conjunctiva; TBC: temporal bulbar conjunctiva; TC: tarsal conjunctiva.

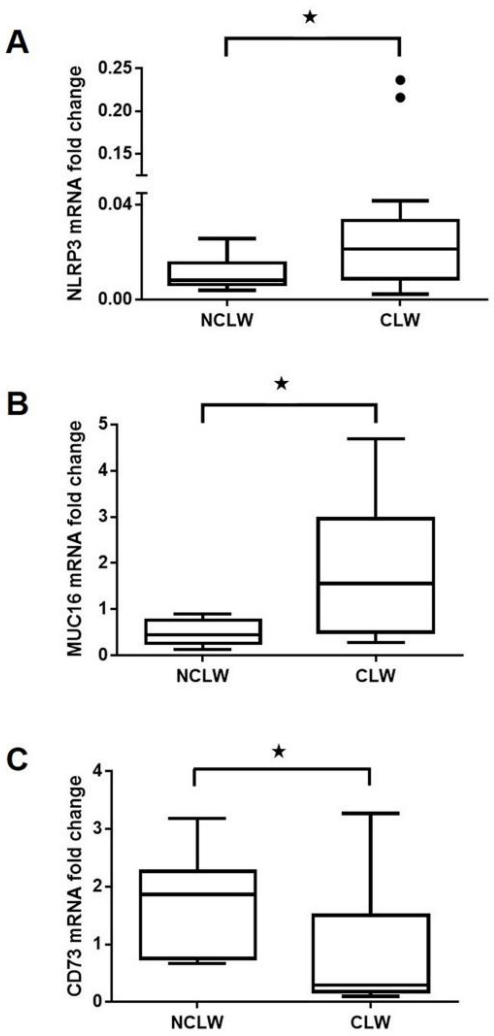


Fig. 1. Impact of contact lens wear on NLRP3 (A), MUC16 (B) and CD73 (C) gene expression in conjunctival cells of middle-aged adults. Gene expression level data for each gene and sample were normalised to HRPT1 signal (internal control). The horizontal line inside the box represents the median value, the lower and upper box lines represent the limits of the interquartile range (25th and 75th percentiles, respectively). Whiskers represent extreme values and dots depict outlier values. * ($p < 0.05$) indicates statistically significant differences between non-contact lens wearers and contact lens wearers. The p values were determined by Mann–Whitney test.

ATP and adenosine, when they reach middle age.

Unlike the genomic effect on CD73, we have observed that CL wear induces MUC16 gene expression. The effect of CL wear on mucin expression has been previously analysed but the results are inconclusive because the studies were conducted without a clearly stated participant age distribution or with age-unmatched between compared groups (Ramamoorthy and Nichols, 2008). MUC16 is a membrane-bound mucin essential to maintain tear film quality and tear film stability, therefore changes in its expression promote ocular surface diseases (Baudouin et al., 2019). In fact, Gipson and colleagues found that postmenopausal women with dry eye disease overexpress MUC16 (Gipson et al., 2011). These authors suggest that this effect is a mechanism to compensate for disease-associated inflammation (Gipson et al., 2011). In this context, some authors have demonstrated that ATP activates NLRP3 inflammasome to induce mucins production in airway epithelial cells (Kim et al., 2018). Furthermore, Paulsen and colleagues found that IL1-β upregulates conjunctival MUC16 mRNA expression (Paulsen et al., 2008). Reciprocally, mucin levels may lead to tear hyperosmolarity that can activate NLRP3 inflammasome (Zheng et al., 2015). All these facts support our results; we have found that long-term CL wear downregulates the CD73 gene (and so the level of ATP can be increased) and upregulates the NLRP3 and MUC16 genes in conjunctival cells of middle-aged wearers.

We have also assessed a potential correlation between these molecular changes and clinical damage. From our results and those of other authors (Chao et al., 2017), it seems that clinical parameters may fail to detect early stages of inflammation in CLW. In this context, we have observed that MUC 16 mRNA level of CLW is inversely proportional to tarsal conjunctiva staining. This result suggests that the expression of MUC16 in ocular surface cells avoids staining. Indeed, Blalock and colleagues have demonstrated this barrier role of MUC16 (Blalock et al., 2008). Consequently, we believe that ocular surface inflammation should be analysed using molecular techniques to detect it early.

All these preliminary results allow us to hypothesise that the long-term use of CL could be a risk factor of ocular surface frailty in middle-aged individuals. Given that frailty predisposes individuals to age-related diseases (Fulop et al., 2010; Hanlon et al., 2018) and that there are more than 140 million CLW worldwide (Stapleton et al., 2007), this is an essential issue. Indeed, Chen and colleagues have observed that a high rate of CLW developed glaucoma and retinal diseases during their CL wearing experience (Chen et al., 2020).

In summary, our gene expression findings suggest that long-term CL wear could trigger an inflammatory response orchestrated by NLRP3 inflammasome and modulated by CD73 and MUC16 proteins in middle age. Further studies are needed to confirm our findings at the protein level as well as to investigate the potential role of long-term CL wear in the onset of ocular frailty.

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Declaration of interest

None.

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Review

Neutrophils and neutrophil extracellular trap components: Emerging biomarkers and therapeutic targets for age-related eye diseases

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ABSTRACT

Age-related eye diseases, including dry eye, glaucoma, age-related macular degeneration, and diabetic retinopathy, represent a major global health issue based on their increasing prevalence and disabling action. Unraveling the molecular mechanisms underlying these diseases will provide novel opportunities to reduce the burden of age-related eye diseases and improve eye health, contributing to sustainable development goals achievement. The impairment of neutrophil extracellular traps formation/degradation processes seems to be one of these mechanisms. These traps formed by a meshwork of DNA and neutrophil cytosolic granule proteins may exacerbate the inflammatory response promoting chronic inflammation, a pivotal cause of age-related diseases. In this review, we describe current findings that suggest the role of neutrophils and their traps in the pathogenesis of the above-mentioned age-related eye diseases. Furthermore, we discuss why these cells and their constituents could be biomarkers and therapeutic targets for dry eye, glaucoma, age-related macular degeneration, and diabetic retinopathy. We also examine the therapeutic potential of some neutrophil function modulators and provide several recommendations for future research in age-related eye diseases.

1. Introduction

Consistent with the population aging and life expectancy prospects (United Nations, 2019), gerontologists expect a high prevalence of age-related conditions, including eye diseases (Akpek and Smith, 2013; Burch et al., 2014). Additionally, chronic eye conditions are the leading cause of visual impairment and blindness worldwide (GBD, 2019 Blindness and Vision Impairment Collaborators and VLEG, 2021). Given that visual loss is associated with other physical/mental disorders, and with an age-related disability, this fact is a critical issue (Court et al., 2014; National Academies of Sciences et al., 2016). Therefore, researchers are dedicating full efforts to understand the mechanisms that lead to chronic ocular diseases onsets.

1.1. Aging and age-related ocular changes

The Aging process involves a progressive decline of each organ-specific functions, including the eye. Like in the rest of the body, age-related ocular changes are caused by oxidative and inflammatory cellular damage (Crooke et al., 2017; De la Fuente and Miquel, 2009).

Abbreviations: ACPAs, Anti-citrullinated protein antibodies; ACPA-IC, Immune complexes formed by ACPAs and citrullinated proteins; AMD, Age-related macular degeneration; APCs, Antigen-presenting cells; AREDs, Age-related eye diseases; DED, Dry eye disease; DR, Diabetic retinopathy; eDNA, Extracellular DNA; FPR, Formyl peptide receptor; LCN2, Lipocalin 2; MGD, Meibomian gland dysfunction; MMP-9, Matrix metalloproteinase-9; MPO, Myeloperoxidase; NE, Neutrophil elastase; NET, Neutrophil extracellular trap; NLR, Neutrophil-to-lymphocyte ratio; oBRB, Outer blood-retinal barrier; oGVHD, Ocular graft-vs-host disease; PACG, Primary angle-closure glaucoma; POAG, Primary open-angle glaucoma; RPE, Retinal pigment epithelium; SII, Systemic immune-inflammation index; TM, Trabecular meshwork.

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Table 1
Summary of the main changes that ocular cells, tissues, systems, and fluids involved in AREDs suffer during aging.

Associate disease	Cells/ Tissues/ Systems	Age-related change	Result	References
DED	Lacrimal gland	Inflammation. Increased amount of lipofuscin granules and oxidative lipid and DNA damage in acinar cells. Lymphocytic infiltration, acinar atrophy, periacinar and periductal fibrosis, ductal dilatation. Decreased peroxidase secretion, lacrimal gland nerves damaged, and neurotransmitter release impaired	Dysfunction of the lacrimal gland. Reduction of the volume and modification of tear film composition (ocular surface damage can subsequently occur)	(De Souza et al., 2019; Kojima et al., 2012; Ríos et al., 2005)
	Meibomian glands	Decreased density and diameter of acinar units. Reduced meibocyte differentiation and cell cycling. Acinar atrophy. Decreased lipid synthesis	Dysfunction of the meibomian glands. Alteration of tear film composition, tear film evaporation (ocular surface damage can subsequently occur)	(Nien et al., 2011; Wei et al., 2011)
	Ocular surface immune system	Increased number and response of Th17 cells. Increased number of dendritic cells able to prime Th1 cells. Increased number of Th1 cells. Decreased amount of Treg cells	Dysregulation of the ocular immune system. Amplification of the ocular surface inflammation. Promotion of corneal epithelial barrier disruption	(Bian et al., 2019; Farid et al., 2016; Foulsham et al., 2020)
	Ocular surface cells	Decreased number of corneal stromal and endothelial cells. Enhanced expression of corneal epithelial TGF-β gene and senescence-associated genes. Decreased corneal stromal nerve density. Reduced number and function of Goblet cells. Expression of conjunctival apoptosis-related genes and inflammation-associated genes	Corneal thinning and reduction of the corneal sensitivity. Ocular surface inflammation and cell death	(Di Zazzo et al., 2019; Giebel et al., 2005; Li et al., 2016; Taurone et al., 2020; Wei et al., 2011; Zhu et al., 2010)
	Tear fluid	Altered protein profile (e.g., increased expression of the inflammatory transcription factor NF-κB)	Amplification of the inflammatory damage	(Nättinen et al., 2019)
Glaucoma	Trabecular meshwork	Decreased number of trabecular meshwork cells and trabecular meshwork stem cells. Reduced trabecular meshwork height. Declined superoxide dismutase activity in the trabecular meshwork. Increased number of oxidized proteins and decreased proteosome activity. Increased expression of cellular senescence markers	Reduction of aqueous humor outflow. Elevation of the intraocular pressure. Reactive oxygen species accumulation and subsequent oxidative damage amplification. Amplification of the inflammatory damage	(Caballero et al., 2004; Choi et al., 2020; De La Paz and Epstein, 1996; Grierson and Howes, 1987; Sundaresan et al., 2019)
	Schlemm's canal	Reduced number and size of giant vacuoles. Decreased number of intracellular pores	Reduction of aqueous humor outflow. Elevation of the intraocular pressure	(Boldea et al., 2001)
	Ciliary muscle	Decreased mobility of the ciliary muscle	Reduction of aqueous humor drainage through trabecular meshwork and generation of optic nerve tension	(Croft et al., 2017)
	Extracellular matrices	Increased stiffening in the trabecular meshwork, lamina cribrosa, sclera, cornea, retina, and Bruch membrane/choroid by an aberrant accumulation of extracellular matrix proteins	Elevation of the intraocular pressure	(Liu et al., 2018)
	Aqueous humor fluid	Altered composition of the aqueous humor (increased expression of inflammatory-related genes) and dynamics (aqueous humor drainage reduced)	Amplification of the inflammatory damage. Intraocular pressure elevation	(Gobelt and Kaufman, 2005; Klein et al., 1992; Toris et al., 1999; Zheng et al., 2018)
	Inner retina and optic nerve head	Increased vulnerability and lowered recovery from injured retinal ganglion cells. Elevated axonal mitochondrial dysfunction. Altered brain-derived neurotrophic factor downstream pro-survival cell signaling, elevated amount of amyloid-β peptide	Amplification of the oxidative damage. Cell death/inner retina thinning. Structural and function deterioration of the inner retina	(Carelli et al., 2004; Gupta et al., 2014; Navarro and Boveris, 2008; Wang et al., 2007)
Glaucoma, DR, AMD	Retina	Induced retinal cells senescence (e.g., retinal ganglion cells, photoreceptor cells, RPE cells, endothelial cells, glial cells, etc.). Dysfunction of Müller cells by lipid peroxidation. Altered expression of calcitonin receptor gene, aquaporins genes and growth factors genes/proteins (e.g., VEGF and PEDF)	Amplification of the inflammatory, oxidative, and vascular damage	(Chen et al., 2010; Curtis et al., 2011; Lee et al., 2021; Nag et al., 2011; Ortak et al., 2013; Smith and Steinle, 2007)
DR	Inner retina	Partial or complete degenerated capillary endothelial and pericytes cells, and arteries/arterioles vascular smooth muscle cells (increased lipofuscin deposits). Changes possibly mediated by lipid peroxidation	Cell death. Cellular senescence. Amplification of the vascular damage	(Nag et al., 2019)
AMD	Outer retina and choroid	Increased deposits of amyloid-β peptide and lipofuscin. Decreased melanin granules and increased lipofuscin deposits in RPE cells. Altered expression at protein and transcript level in RPE cells (e.g., decreased expression of antioxidant enzymes, and increased gene expression of P53 and its targets genes that mediated apoptosis, senescence, and innate immune response). Increased thickening and stiffening of the Bruch membrane. Decreased thickening of the choroid and upregulated proinflammatory choroid signaling. Periphery hard drusen formation. Elevated vulnerability of photoreceptor cells. Altered microglial genes expression, compromising the supportive function of these cells, and altering their activation	Cell death. Amplification of the oxidative, inflammatory, and vascular damage	(Chaum et al., 2015; Gu et al., 2012; Gupta et al., 2017; Liu et al., 2018; Ma et al., 2013; Nag, 2021; Ratnayaka et al., 2015; Robbie et al., 2016)

This damage triggers changes in the different structures, and thus, it alters eye physiological function. For example, the formation of protein aggregates (induced by age-related oxidative stress) in the lacrimal glands causes premature senescence (increases the synthesis and secretion of inflammatory mediators) that impair their function, reducing tear secretion (Alves et al., 2005; Ríos et al., 2005; Yoon et al., 2020). This reduction evokes damage to the corneal epithelial cells, which can result in an irregular corneal surface, affecting visual quality (De Paiva, 2017). Likewise, the formation of those aggregates in the extracellular matrix of trabecular meshwork cells, retinal capillary endothelial cells, or retinal pigmented epithelial cells, increases intraocular pressure and breaks blood-retinal barriers that can also lead to a vision loss (Anand-Babu et al., 2019; Murali et al., 2020; Park and Kim, 2012; Sharif et al., 2019; Tektas and Lütjen-Drecoll, 2009; Xu et al., 2018).

Moreover, the continued exposure to solar UV radiation, and the postmitotic character of ocular cells, make the eye extremely vulnerable to age-related oxidative damage (Crooke et al., 2017). Ocular cells also become senescent during the aging process secreting pro-inflammatory mediators that damage the surrounding cells and attract immune cells (Park and Kim, 2012; Prata et al., 2018; Rocha et al., 2020; Shosha et al., 2018; Sun et al., 2018). Similarly, immune cells undergo age-related functional changes that lead to a high inflammatory environment (Fulop et al., 2017; Mashaghi et al., 2017). So, ocular non-immune senescent cells and aged immune cells generate and prolong the inflammatory cellular/tissular damage. Some authors have suggested that age-related disease onsets are due to accelerated/intensive aging (Franceschi et al., 2018). Indeed, cells and tissues involved in age-related eye diseases (AREDs) suffer more accelerated/intensive changes during illness than aging (Feher et al., 2006; Foulsham et al., 2020; Gabelt and Kaufman, 2005; Kojima et al., 2012; Lamoke et al., 2015; Lin et al., 2011; Tezel et al., 2007; Fasanella et al., 2016). The knowledge of these changes can provide valuable information to slow aging and thus prevent AREDs. Table 1 summarizes the main age-related changes in cells, tissues, systems, and fluids involved in AREDs.

1.2. Neutrophils and neutrophil extracellular traps

Neutrophils are one of the immunological cells that undergo functional changes during aging (Alonso-Fernández et al., 2008; Drew et al., 2018). Indeed, some authors have suggested that the host environment (e.g., age-associated inflammatory milieu) could induce expression changes in neutrophils, which might affect their cellular functions (Drew et al., 2018). In this sense, *in vitro* studies have demonstrated that neutrophils from aged individuals present lower chemotactic and phagocytic activity and produce a higher amount of intracellular reactive oxygen species than cells from young individuals (Alonso-Fernández et al., 2008; Wenisch et al., 2000). Moreover, *in vivo* neutrophil trafficking of older animals is also altered (Barkaway et al., 2021). Neutrophils from these animals reverse transendothelial migration (which allows their biological clearance) and return to circulation, causing distant tissue damage (Barkaway et al., 2021). This abnormal trafficking plays a pivotal role in both innate and adaptive immune responses. Thus. neutrophils mediate innate immunity –the first line of defense against pathogens– but are also involved in adaptive immunity as modulators of T cells, and B cells functions (Li et al., 2019; Mayadas et al., 2014). The interface between these immune functions is the termed NETosis process (Brinkmann et al., 2004; Delgado-Rizo et al., 2017; Mayadas et al., 2014). In this process, neutrophils extrude a meshwork of nuclear/mitochondrial DNA (extracellular DNA or eDNA) and cytosolic granule peptides/proteins (Brinkmann et al., 2004; Yousefi et al., 2009) (Fig. 1). Given that this meshwork serves to immobilize and kill pathogens, it is known as Neutrophil Extracellular Trap (NET). Nevertheless, the presence of these neutrophils constituents in the extracellular medium is also critical to activate adaptive immune cells and thus the inflammatory response (Delgado-Rizo et al., 2017; Li et al., 2019; Mayadas et al., 2014). For example, the neutrophil peptide LL-37 (cathelicidin) forms a complex with eDNA that promotes the synthesis of interferon-alpha (IFN-α), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNFα) from dendritic cells (Delgado-Rizo et al., 2017; Li et al., 2019; Minns et al., 2019). These cytokines activate dendritic cells and so indirectly stimulate the proliferation of T cells (Delgado-Rizo et al., 2017; Li et al., 2019). Other neutrophil proteins – myeloperoxidase (MPO), neutrophil elastase (NE), lipocalin 2 (LCN2), citrullinated histones, and peptidyl-arginine deiminases (PADs) – also have an immunomodulatory role (Cassatella et al., 2019; Floderer et al., 2014; Minns et al., 2019; Mowen and David, 2014; Zhou et al., 2017). Interestingly, PADs are crucial for NETs formation and are pathologic mediators of chronic inflammation (Mowen and David, 2014; Zhou et al., 2017). These enzymes catalyze the citrullination of proteins that results essential for the NET formation (via histones citrullination) and leads to the formation of autoantibodies (anti-citrullinated protein antibodies, ACPAs) (Mowen and David, 2014). Additionally, citrullinated histones induce cell death, supporting inflammatory damage (Fig. 1) (Saffarzadeh et al., 2012; Silvestre-Roig et al., 2019). The extracellular concentration of these proteins and the presence/absence of other local inflammatory mediators determine the pro-inflammatory or anti-inflammatory outcome (Cassatella et al., 2019; Floderer et al., 2014; Minns et al., 2019). Furthermore, some inflammatory mediators (e.g., IL-6, IL-1β, IL-8, TNF-α, and ACPAs) stimulate the NETosis process, and thus, they increase the extracellular concentration of NET proteins (Delgado-Rizo et al., 2017; Sur Chowdhury et al., 2014). Consequently, in a high inflammatory situation like aging or age-related diseases, neutrophils and their nuclear/cytosolic components could exacerbate the inflammatory damage.

Few studies (and mostly animal studies) have evaluated the impact of age on the NETosis process (Hazeldine et al., 2014; Lu et al., 2021; Ortmann and Kolaczkowska, 2018). In this context, Lu et al. have recently demonstrated that unprimed neutrophils from aged mice present a higher NETosis inducibility than young ones, particularly in female animals (Lu et al., 2021). Furthermore, these authors have observed that a priming process for NET formation, autophagy, is upregulated in aged neutrophils (Lu et al., 2021). Impairment of autophagy is also associated with inflammatory and age-related diseases, including ocular ones (Cheon et al., 2019; Crooke et al., 2017). Consequently, NETosis alteration could also play a role in age-related eye diseases (AREDs) onsets.

2. Neutrophils, neutrophil extracellular trap components and age-related eye diseases

AREDs including dry eye, glaucoma, age-related macular degeneration, and diabetic retinopathy, share underlying oxidative and inflammatory molecular processes common to aging and other age-related diseases (Crooke et al., 2017; Dogru et al., 2018; Ganesalingam et al., 2019; Johnson et al., 2015). One of these shared mechanisms seems to be the impairment of NETs formation/degradation processes. AREDs patients present local recruitment of neutrophil cells and overexpression of neutrophil extracellular trap components at both, local and systemic levels (An et al., 2019; Arafat et al., 2017; Aragona et al., 2006; Chau et al., 2007; Chen et al., 2020; Chung et al., 2016; Ghosh et al., 2019, 2017; Guo et al., 2011; Kaeslin et al., 2016; Park et al., 2016; Reyes et al., 2018; Sonawane et al., 2012; Tibrewal et al., 2013; Ueno et al., 2018; Wang et al., 2018). In this context, the neutrophil-to-lymphocyte ratio (NLR), a blood indicator of systemic inflammation and poor diseases prognosis (Azab et al., 2012; Bian et al., 2010; Jin et al., 2013), has also been studied and validated as AREDs biomarker (Ilhan et al., 2015; Kurtul and Ozer, 2016; Kurtul et al., 2016; Niazi et al., 2019; Ozgonul et al., 2016; Sekeryapan et al., 2016; Ulu et al., 2013; Yue et al., 2015).

These data suggest that neutrophils, NETs, and their constituents could be potential diagnostic biomarkers and therapeutic targets of AREDs.

2.1. Dry eye disease

The outer ocular surface comprises a continuous layer of epithelium (corneal and conjunctival epithelium) that provides physical/immunologic protection to the eye and contributes to the proper visual performance (Bron et al., 2017). The tear film covers this epithelium and thus, protects, nourishes, and lubricates it. This film is composed of an inner mucous/aqueous phase produced by lacrimal glands and conjunctival cells (epithelial and Goblet cells), and an external lipid phase generated by Meibomian glands (Bron et al., 2017). Due to different underlying causes, the disruption of this tear film can cause a loss of homeostasis and might result in ocular surface diseases.

According to the Dry Eye Workshop (DEWS II) report published by the Tear Film and Ocular Surface Society (TFOS) in 2017, Dry Eye Disease (DED) is defined as a "multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles." (Craig et al., 2017).

The prevalence of DED established in 2017 varies from approximately 5–50% worldwide, with age considered as one of the most relevant risk factors (Stapleton et al., 2017). Indeed, aging predisposes to DED, and its critical underlying inflammatory and oxidative damage mechanisms are common to aging and age-related diseases (Craig et al., 2017; De la Fuente and Miquel, 2009; Dogru et al., 2018; Franceschi et al., 2007; Seen and Tong, 2018; Sharma and Hindman, 2014). For these reasons, some authors (including us) consider that DED is an age-related disorder (De Paiva, 2017; Farid et al., 2016; Gipson, 2013; Malet et al., 2014; Rusciano et al., 2018; Seen and Tong, 2018). This fact explains the emerging interest in these underlying signaling pathways shared by aging and age-related diseases to find more effective DED biomarkers and therapeutic agents.

Among the different causes for ocular surface inflammation, tear hyperosmolarity is considered a crucial stressor (Bron et al., 2017). Hyperosmolarity results from either aqueous tear deficiency (condition called aqueous-deficient dry eye) or increased evaporation of the aqueous tear phase (disease called evaporative dry eye), or a combination of both (Bron et al., 2009). As a result of hyperosmolarity, a cascade of inflammatory events take place in the ocular surface, which leads to epithelial cell death and alters the stability of the tear film, perpetuating the disease (Bron et al., 2017; Tibrewal et al., 2014). Immunological cells like antigen-presenting cells (APCs) and T lymphocytes sustain this inflammatory damage (Tibrewal et al., 2013). Neutrophils, which can act as APCs and modulate T cell functions (Li et al., 2019), are massively recruited to the ocular surface under non-basal conditions as stress or tissue injury (Gronert, 2010). Besides, *in vitro* hyperosmolar condition promotes the activation of the NETosis process (Tibrewal et al., 2014). Given these facts, Tibrewal et al. have suggested that DED-associated hyperosmolar stress could stimulate the NETosis process from neutrophils recruited in the ocular surface of DED patients (Tibrewal et al., 2014). In this context, various authors demonstrated the high presence of neutrophils (both at the systemic and local level) and NETs components in the tears of DED patients (Fig. 2) (An et al., 2019; Sonawane et al., 2012; Tibrewal et al., 2013).

According to Sekeryapan et al., the NLR values were significantly higher in patients with a non-autoimmune DED than in controls (Fig. 2) (Sekeryapan et al., 2016). Recently, Ozarslan Ozcan et al. have proved that the systemic immune-inflammation index (SII) -which considers neutrophil, platelet, and lymphocyte counts- is also high in patients of this DED type (Ozarslan Ozcan et al., 2020). Besides, these authors claim the superior ability of SII to measure the inflammatory status of DED patients than other inflammatory markers like NLR (Ozarslan Ozcan et al., 2020). Further studies are needed to confirm these results and to evaluate the correlation between NLR/SII and the severity of the disease.

About the local increment of neutrophils in DED patients, Reyes and colleagues have demonstrated that neutrophils promote meibomian gland obstruction in an animal model of chronic inflammation (Reyes et al., 2018). Moreover, these authors found that the tear of patients with meibomian gland dysfunction (MGD) presented high neutrophil levels correlated with MGD severity (Fig. 2) (Reyes et al., 2018). MGD, the most common cause of evaporative dry eye, is a chronic abnormality of the meibomian glands that results in a loss of tear lipids, which boosts tear evaporation leading to tearing film instability and hyperosmolarity (Bron and Tiffany, 2004; Nelson et al., 2011).

Patients with a type of aqueous-deficient dry eye named ocular graft-vs-host disease (oGVHD) also present in their tears high levels of neutrophils correlated with a more severe form of this disease (Fig. 2) (An et al., 2019). This ocular manifestation of the GVHD occurs in 40–60% of patients after allogeneic hematopoietic stem cell transplantation (Shikari et al., 2013). The tear of oGVHD patients also presents high levels of eDNA and NET-specific proteins like NE, MPO, and extracellular matrix metalloproteinases (MMP-9 and MMP-8) (Fig. 2) (An et al., 2019; Arafat et al., 2017; Sonawane et al., 2012). Likewise, the tears and ocular surface of patients with other types of aqueous-deficient DED like Sjögrenś syndrome dry eye, present high levels of NETs and their components (e.g., eDNA and LCN2) (Aragona et al., 2006; Kwon et al., 2020; Tibrewal et al., 2013). More recently, Kwon et al., have demonstrated that the ocular surface wash of both, aqueous-deficient (e.g., oGVHD and Sjögrenś syndrome) and evaporative dry eye (e.g., MGD) patients, contains an abnormal amount of citrullinated proteins, PAD4 enzyme, and ACPAs (Kwon et al., 2020). These ACPAs form complexes with citrullinated proteins that could interact with immune cells (e.g., dendritic cells or neutrophils) and the complement system, leading to the secretion of pro-inflammatory mediators (Kwon et al., 2020). Additionally, these authors have demonstrated that ocular surface ACPAs also stimulate the NETosis process *in vitro*, and in this form, they might prolong the inflammatory cellular/tissular damage (Kwon et al., 2020). In this context, Kwon and coworkers have proved that ACPAs can cause ocular surface disease in mice (Kwon et al., 2020). The ocular surface wash from the closed eye of DED patients also presents an increased number of neutrophils, which are in an enhanced state of degranulation (Postnikoff et al., 2020). Some authors have suggested that this degranulation could promote DED-related inflammatory damage (Postnikoff et al., 2020). Given that the NETosis process seems to play a critical role in DED onset and NETs are also present in the awakened eye (Mahajan et al., 2019), the connection between both processes (degranulation and NETosis) and the DED-intrinsic inflammatory damage could be a fascinating topic for future work.

As we commented above, components like eDNA can trigger the synthesis and release of pro-inflammatory cytokines that amplify the inflammatory damage. In this sense, Sonawane et al. have demonstrated that the conjunctival cells of severe DED patients (e.g., oGVHD ones) overexpress mRNAs encoding for cytosolic DNA sensors and pro-inflammatory cytokines (Sonawane et al., 2012). These results suggest that both neutrophils and the NETosis process play a critical role in severe DED onset. Therefore, the tear eDNA and the NETosis process have been evaluated as potential therapeutic targets of DED (Tibrewal et al., 2014, 2013).

Regarding the eDNA, Sandeep Jain's group tested the efficacy of DNase I eyedrops in two patients with severe DED (Tibrewal et al., 2013). This treatment reduced the tear eDNA abundance and improved the patients' eye comfort. eDNA degradation decreases the inflammation and viscosity of the tears, thus generating comfort. This group have also performed a phase I/II clinical trial with 47 DED patients of which 25 were treated with DNase eyedrops (ClinicalTrials.gov Identifier: NCT02193490). Patients treated with DNase presented a significant lower corneal damage and presence of surface mucoid debris/strands – a mix rich in NETs and their components – than vehicle-treated patients (Fig. 2) (Mun et al., 2019). Moreover, DNase treatment was safe and well-tolerated (Mun et al., 2019). Sandeep Jain's group is now testing the efficacy of pooled ocular surface immunoglobulin (IgG)-eye drops (OSIG-eye drops) in patients with DED (e.g., Sjögrenś syndrome dry eye,

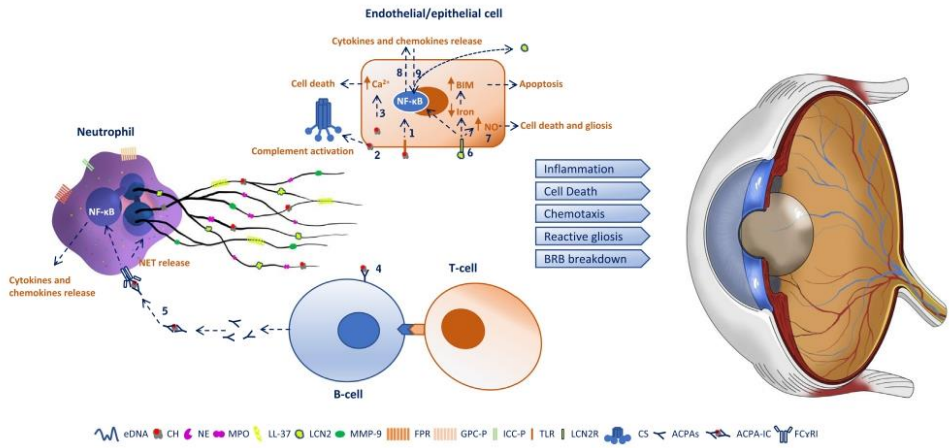


Fig. 1. Schematic representation of the Neutrophil Extracellular Trap (NET) and **possible harmful effects of NET constituents on ocular cells involved in AREDs**. The figure shows a neutrophil with some surface receptors [formyl peptide receptors (FPR), Fcγ receptors I (FcγRI), G protein-coupled purinergic receptors (GPC-P, e.g., P2Y2, P2Y6, A3, and A2A), and ion channel-coupled purinergic receptors (ICC-P, e.g., P2X7)] and NET components [extracellular DNA (eDNA) and components released from neutrophil primary granules (MPO, myeloperoxidase; NE, neutrophil elastase), secondary granules (LL-37, cathelicidin; LCN2, lipocalin 2), and tertiary granules (MMP-9, matrix metalloproteinase-9)]. NET components can cause inflammation, cell death, chemotaxis, reactive gliosis, and BRB breakdown on eye tissues of AREDs patients. The picture illustrates possible cell damage mechanisms evoked by NETs components in the eye: extracellular citrullinated histones (CH) activate (1) toll-like receptors (TLR), driving the NF-κB-mediated transcription of pro-inflammatory cytokines, and (2) the complement system (CS). CH can also penetrate the plasma membrane, resulting in an influx of Ca^{2+} that leads to cell death (3). B-cells also recognize CH and, after T-cells stimulation, make anti-citrullinated protein antibodies (ACPAs). ACPAs can form immune complexes with citrullinated proteins (ACPA-IC) (5) that activate FcγRI of neutrophils and dendritic cells. This activation finally leads to the release of pro-inflammatory cytokines and NETs. Extracellular LCN2 binds to LCN2 receptors (LCN2R) that mediates its uptake via receptor-mediated endocytosis (6). Intracellular LCN2R can expel iron outside the cell, which induces the expression of the pro-apoptotic protein Bim. Intracellular LCN2 induces NO formation, promoting cell death and gliosis (7), and triggers the synthesis and release of cytokines and chemokines (8). Conversely, pro-inflammatory mediators evoke the synthesis and release of more LCN2 from local cells (9). For a more detailed review of CH and LCN2 harmful cell effects, see (Silk et al., 2017) and (Jha et al., 2015), respectively.

oGVHD, and MGD patients) (ClinicalTrials.gov Identifier: NCT04510428) (Kwon et al., 2020). These immunoglobulins are present in an unusual amount in the ocular surface wash of these patients, and Kwon et al. have shown their ability to reverse *in vitro* the ACPAs-induced NETosis (Kwon et al., 2020). A preliminary study with 27 DED patients has confirmed the efficacy and safety of this treatment (Kwon et al., 2020). OSIG-eye drops diminish the level of neutrophilic and NET biomarkers, and clinically, they reduce the signs and symptoms of DED (Kwon et al., 2020). These encouraging study provides the scientific justification for advancing to phase III clinical trials.

Tibrewal et al. also investigated the usefulness of direct/indirect NETosis inhibitors under a hyperosmotic condition like the one found in DED (Tibrewal et al., 2014). Among them, annexin-1 peptide and lipoxin A4 were the most effective inhibitors causing a significant decrease of the NETosis process in *ex vivo* neutrophils exposed to hyperosmolar stress (Fig. 2) (Tibrewal et al., 2014). These anti-inflammatory/pro-resolution mediators are agonists of the neutrophil surface formyl peptide receptor 2 (FPR2) (Dorward et al., 2015). The interaction of these agonists with FPR2 limits the neutrophil recruitment to the inflammation site and induces their apoptosis in this site (Dorward et al., 2015; Sugimoto et al., 2016). Consequently, annexin 1 peptide and lipoxin A4 could also have a therapeutic value in DED patients, acting as indirect anti-inflammatory agents that limit the NETosis process and promote the release of anti-inflammatory molecules on the ocular surface.

Annexin 1 peptide can also regulate neutrophil migration acting on its formyl peptide receptor 1 (FPR1) (Dorward et al., 2015). This peptide activates FRP1 that evokes autocrine release of adenosine triphosphate (ATP). Subsequently, neutrophil purinergic receptors like $P2Y_2$ and adenosine A3 receptors are activated by ATP and adenosine (nucleoside derived from the breakdown of extracellular ATP), modulating their migratory activity (Dorward et al., 2015). Interestingly, clinical studies supported the use of purinergic receptor agonists for DED therapy (Avni et al., 2010; Ohashi et al., 2020), although their role in the ocular surface recruitment of neutrophils is unknown (Fig. 2).

Given the intricate molecular interactions underlying DED pathogenesis (Bron et al., 2017; Ganesalingam et al., 2019), the combination therapy approach appears more suitable than the single-agent treatment to achieve long-term benefit in DED patients. This approach is a common practice in ophthalmology to treat diseases with complex pathogenesis (Holló et al., 2014; Peyman and Hosseini, 2011). In this context, Tibrewal and colleagues have proposed using a drug cocktail constituted by pro-resolutive, osmoprotective, and DNase 1 agents for DED treatment (Tibrewal et al., 2014).

Further studies are needed to validate the therapeutic potential of all these strategies for treating dry eye disease.

2.2. Glaucoma

Glaucoma is an optic neuropathy characterized by the progressive neurodegeneration and death of retinal ganglion cells and the subsequent damage to the optic nerve (Quigley, 2011). This damage causes a gradual loss of vision starting in the peripheral parts of the visual field, that leads to partial or total blindness if untreated. This disease is the second leading cause of visual impairment and blindness worldwide (GBD, 2019 Blindness and Vision Impairment Collaborators and VLEG, 2021). Despite the neurodegeneration that occurs in the retina, glaucoma is caused by impairment of aqueous humor outflow through the drainage structures found in the iridocorneal angle, the trabecular meshwork (TM) and the canal of Schlemm. Anatomically normal

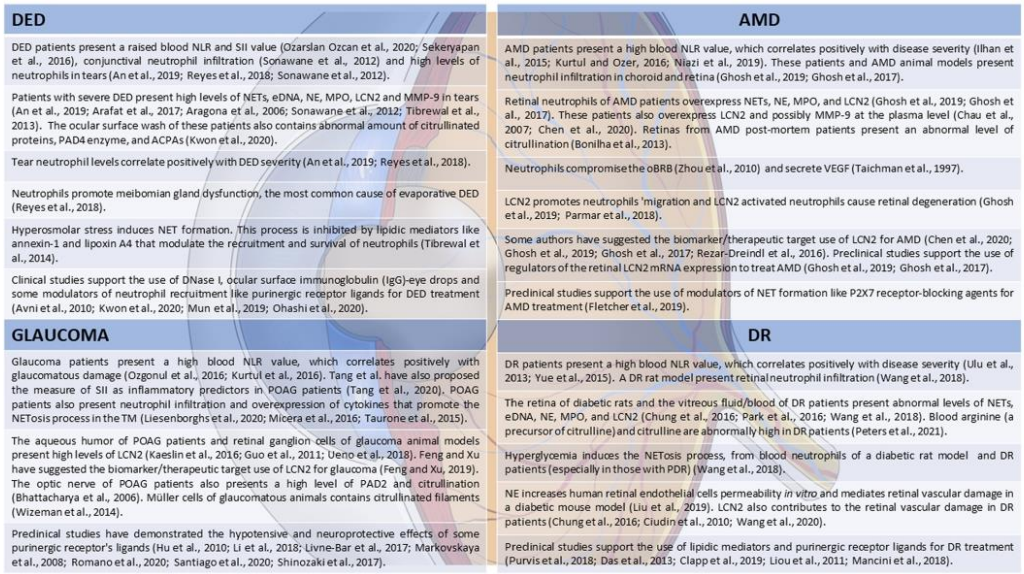


Fig. 2. Overview of the link between neutrophils/NETs components and age-related eye diseases. AMD, Age-related macular degeneration; eDNA, extracellular DNA; DED, dry eye disease; DR, Diabetic retinopathy; LCN2, lipocalin 2; MMP-9, matrix metalloproteinase-9; MPO, myeloperoxidase; NE, neutrophil elastase; NETs, neutrophil extracellular traps; NLR, neutrophil-to-lymphocyte ratio; oBRB, blood-retinal barrier; PAD4, peptidyl-arginine deiminase 4; POAG, primary open-angle glaucoma; SII, systemic immune-inflammation index; TM, trabecular meshwork; VEGF, vascular endothelial growth factor.

structures are found in the most frequent cause of glaucoma, primary open-angle glaucoma (POAG), that has a global prevalence of 3.1% (Tham et al., 2014). In this form of glaucoma, functional alterations in the outflow tissues are thought to reduce the outflow of aqueous humor towards the aqueous veins, thus reducing outflow and increasing intraocular pressure, the main risk factor of developing the retinal degeneration associated to this disease. A second common form of glaucoma is primary angle-closure glaucoma (PACG), with a prevalence of 0.5% in the general population (Tham et al., 2014). In this case, a partial or total impairment of aqueous humor outflow is found, together with the associated rise in intraocular pressure. This is due to an anatomically narrow iridocorneal angle or the partial or total blockade of this structure due to different problems, such as a forward displacement of the iris towards the cornea, narrowing the anterior chamber of the eye.

Given that the glaucoma prevalence increases exponentially in people older than 40 years, age is considered a key risk factor for POAG and PACG onset (Caprioli, 2013; Cheng et al., 2014; Quigley, 2011; Tham et al., 2014). In this context, various authors have demonstrated the age-related loss of trabecular meshwork cells and retinal ganglion cells that are particularly important in glaucoma (Balazsi et al., 1984; Grierson and Howes, 1987; Harman et al., 2000). Likewise, some studies have reported that these cells become susceptible to oxidative damage and senescent with age (De La Paz and Epstein, 1996; Park and Kim, 2012; Wang et al., 2007). These age-related changes also occur in glaucoma patients who seem to experiment an accelerated aging (Alvarado et al., 1984; Caprioli, 2013; Rocha et al., 2020; Tezel et al., 2007). As in all age-related diseases, oxidative stress (mainly at mitochondrial level) plays an essential role in glaucoma onset and progression (reviewed in (Crooke et al., 2017)). In addition to age, other factors such as high intraocular pressure (IOP) (the only widely accepted modifiable glaucoma risk factor) (Blumberg et al., 2015) contribute to oxidative stress-induced mitochondrial damage (Kong et al., 2009). As mentioned earlier, this elevated IOP is due to an imbalance between the aqueous humor production across the ciliary body and its drainage through the TM that decreases with age and disease (De Groef et al., 2016; Gabelt and Kaufman, 2005). Additionally, high IOP results in retinal glial cell activation (Inman and Horner, 2007; Ju et al., 2006; Woldemussie et al., 2004). Therefore, activated glial cells that release immunomodulatory mediators (e.g., TNF- α and NO) and trabecular/retinal ganglion senescent cells that secrete pro-inflammatory mediators, contribute to the chronic inflammatory process characteristic of glaucoma (Crooke et al., 2017; Neufeld, 1999; Park and Kim, 2012; Prata et al., 2018; Rocha et al., 2020; Tezel, 2011; Tezel and Wax, 2000). In this sense, some authors have demonstrated that the TM of POAG patients recruits neutrophils and overexpresses pro-inflammatory cytokines (such as TNF-α, IL-1β, and IL-6) that promote the NETosis process (Fig. 2) (Liesenborghs et al., 2020; Micera et al., 2016; Taurone et al., 2015). Similarly, the aqueous humor of POAG patients presents a high level of the NET component LCN2 (Fig. 2) (Kaeslin et al., 2016). Kaeslin et al. have claimed the role of innate immunity and particularly neutrophils in glaucoma onset as the source of this LCN2 (Kaeslin et al., 2016). However, other cells can also release this protein, contributing to the total pool of POAG patients' aqueous humor LCN2. Interestingly, Liu et al. have found that the TM of POAG patients down-express LCN2 gene (Liu et al., 2013). Therefore, more research is needed to find other cellular sources of this aqueous humor LCN2. POAG patients could also overexpress LCN2 in their retinal ganglion cells as occurs in glaucomatous rats and mice models (Guo et al., 2011; Ueno et al., 2018). Neutrophil cells release LCN2, which in turn regulates their migration to the inflammation site (Jha et al., 2015). Retinal cells such as microglial, astrocyte, and ganglion cells can also release LCN2, which can act over them, regulating the neuroinflammation process (Jha et al., 2015). Indeed, Feng and Xu have suggested the involvement of LCN2 in glaucoma pathogenesis and its potential role as a biomarker/therapeutic target against this disease (Fig. 2) (Feng and Xu, 2019). The optic nerve

of POAG patients also presents a high level of PAD2 and citrullination (Bhattacharya et al., 2006). Interestingly, Bhattacharya et al. have suggested that elevated IOP could induce PAD2 expression (Bhattacharya et al., 2006). Given that arterial hypertension evokes *ex vivo* NETosis (Hofbauer et al., 2017), high IOP might also promote NETosis releasing more PAD2. Müller cells (a type of retinal glial cells) of a mouse model of glaucoma also express citrullinated filaments, which could mediate pathological gliosis (Wizeman et al., 2014).Therefore, citrullination could play a role in POAG, leading to retinal and optic nerve damage (Bhattacharya et al., 2006; Wizeman et al., 2014).

In this context, Ozgonul et al. have proposed the NLR parameter as a diagnostic biomarker of POAG (Ozgonul et al., 2016). The authors found an abnormally high value of the NLR parameter in POAG patients, which correlated well with a high visual field defect (glaucomatous damage) (Fig. 2) (Ozgonul et al., 2016). Recently, Tang et al. have confirmed the validity of this parameter and proposed the measure of SII as inflammatory predictors in POAG patients (Tang et al., 2020). Similarly, patients with pseudoexfoliation glaucoma present a remarkably high NLR value (Kurtul et al., 2016). However, other authors found no difference in NLR between subjects with normal-tension glaucoma and the control group (Atalay et al., 2019).

Altogether, these results suggest that neutrophils and probably the NET components play a role in glaucoma onset and could be biomarkers and therapeutic targets for this disease. In this sense and like in dry eye disease, some preclinical studies have suggested the anti-inflammatory and neuroprotective role of annexin-1 and lipoxin A4 (indirect NETosis inhibitors) in experimental glaucoma (Fig. 2) (Li et al., 2018; Livne-Bar et al., 2017).

Similarly, preclinical works have proved the hypotensive and neuroprotective effects of different purinergic receptor ligands (Fig. 2) (Fonseca et al., 2017; Markovskaya et al., 2008; Romano et al., 2020; Santiago et al., 2020; Shinozaki et al., 2017; Soto et al., 2005). So, the activation of purinergic receptors like $P2Y_1$ and $P2Y_6$ reduces IOP (Fonseca et al., 2017; Markovskaya et al., 2008; Soto et al., 2005). These effects are possible because cells involved in glaucoma pathogenesis (e.g., ciliary cells, trabecular meshwork cells, retinal ganglion cells, and glial cells) express these receptors on their surface (Crooke et al., 2008; Fonseca et al., 2017; Markovskaya et al., 2008; Soto et al., 2005). Indeed, the dysregulation of these receptors in animals results in glaucomatous optic neuropathy (Hamada et al., 2021; Shinozaki et al., 2017). In this sense, various authors have demonstrated the role of glial $P2Y_1$ and $P2Y_6$ receptors in neuroinflammation (Kita et al., 2019; Yang et al., 2017). However, this neuroprotective effect could be also due to neutrophil $P2Y_1$/$P2Y_6$ receptors that are involved in neutrophil activation and recruitment, and NET formation (Grbic et al., 2012; Sil et al., 2017; Wang and Chen, 2018). Likewise, the $P2X_7$ receptor, which is also present on the surface of retinal ganglion cells, glial cells, and immune cells, seems to be involved in glaucoma-related neuroinflammation (Fletcher et al., 2019). The ATP-activated receptor mediates retinal ganglion cell death (Hu et al., 2010; Romano et al., 2020). Moreover, the activation of $P2X_7$ receptors can lead to pannexin-1 channel-dependent ATP release in all cells mentioned contributing to retinal ganglion cell death (Fletcher et al., 2019). Thus, neutrophils that express both $P2X_7$ receptor and pannexin-1 channel could mediate ganglion cell death but also the secretion of pro-inflammatory mediators and NETosis process in the glaucomatous retina (Pelegrin and Surprenant, 2009; Sofoluwe et al., 2019). Further studies are needed to confirm the therapeutic potential of all these molecules for treating glaucoma.

2.3. Age-related macular degeneration

Age-related macular degeneration (AMD) is a progressive degenerative disease of the outer retina and the third leading cause of vision loss worldwide (GBD, 2019 Blindness and Vision Impairment Collaborators and VLEG, 2021). Although age is the principal risk factor for AMD, other factors such as genetic predisposition, environmental and behavioral factors may also increase the risk of its onset (Mitchell et al., 2018). Aged-related retinal changes, mainly over retinal pigment epithelial and retinal glial cells, generate a favorable environment to AMD onset (Crooke et al., 2017; Telegina et al., 2018). Besides, many of these changes are exacerbated in AMD, promoting oxidative damage and the subsequent immune-inflammatory, drusen formation, and neovascularization events characteristic of this disease (Ardeljan and Chan, 2013; Nowak, 2013; Telegina et al., 2018). Drusen are extracellular deposits (rich in photooxidative products) associated with the early/intermediate AMD and localized beneath retinal pigment epithelium (RPE), impairing its metabolic connection with the choroid (Crooke et al., 2017; Mitchell et al., 2018). The choroidal neovascularization or growth of new frail vessels is associated with late AMD and is the principal cause of severe vision loss (Mitchell et al., 2018; Telegina et al., 2018).

Regarding the immune-inflammatory events (e.g., the complement system activation, the change to senescent RPE cells, and the exacerbated activation of glial cells) are also involved in drusen formation and choroidal neovascularization events (Bradley et al., 2011; Jensen et al., 2020; Marazita et al., 2016; Telegina et al., 2018; Wang et al., 2019). Like in glaucoma, AMD retinas present an abnormal level of citrullination (Bonilha et al., 2013). Nevertheless, non-AMD and AMD post-mortem patients express similar levels of retinal PAD2 (Bonilha et al., 2013). These data suggest that AMD retinas present a reduced rate of turnover of citrullinated proteins, contributing to AMD-associated adverse events.

As mentioned above, LCN2 can regulate the neuroinflammation process present in age-related retinal pathologies. Given that LCN2 receptors are present in the surface of retinal cells like glial and RPE cells, this protein can modulate their functions (e.g., glial activation and RPE survival) and thus the inflammatory response (Ghosh et al., 2020; Parmar et al., 2018; Tang et al., 2018). In this sense, the retina of AMD patients overexpresses LCN2 (Fig. 2) (Ghosh et al., 2019, 2017). Ghosh and coworkers have proved that the choroid and retina of early AMD patients have a higher infiltration of LCN2 expressing neutrophils than age-matched controls (Ghosh et al., 2019, 2017, 2020). These neutrophils also express other NET proteins such as MPO and NE (Fig. 2) (Ghosh et al., 2019, 2017). Furthermore, Ghosh et al. have insinuated that these infiltrated cells could also overexpress MMP-9, which may form a complex with LCN2 in the outer retina-choroid of AMD patients (Ghosh et al., 2020). Therefore, the LCN2/MMP-9 complex could facilitate AMD choroidal vascularization as seems to occur in corneal vascularization (Shen et al., 2016).

Neovascularization may occur after the outer blood-retinal barrier (oBRB) breakdown and the subsequent recruitment of circulating immune cells like neutrophils, which, together with senescent RPE cells, secrete vascular endothelial growth factor (VEGF) and promote the growth of new vessels (Marazita et al., 2016; Taichman et al., 1997). Therefore, neutrophil-derived MMP-9 could compromise oBRB, formed by the tight junctions between adjacent RPE cells (Zhou et al., 2010), and permit the pass of systemic immune and inflammatory components into the retina, amplifying the vascular damage and neuroimmune response (Yang et al., 2020). In this context, a current phase II clinical trial tries to evaluate the efficacy of an MMP-9 inhibitor against recalcitrant neovascular AMD (ClinicalTrials.gov Identifier: NCT04504123). Moreover, Chau et al. found a high plasma level of MMP-9 in subjects with AMD (Fig. 2) (Chau et al., 2007). Nevertheless, a recent study has proved that neovascular AMD patients only overexpress LCN2, but not MMP-9 or LCN2/MMP-9 complex, in their plasma (Fig. 2) (Chen et al., 2020). Therefore, the potential expression of MMP-9 on retinal neutrophils and their role in AMD vascular and neuroinflammatory damage is an exciting topic to explore in future research.

Regarding the potential role of LCN2 in AMD damage, this NET protein also regulates neutrophils' migration into the retina, where in conjunction with retinal cells (mostly RPE cells), synthesize and release new LCN2, potentiating outer retinal degeneration (Ghosh et al., 2019).

Parmar et al. have suggested that the compromised oBRB may contribute to leakage of LCN2 produced in the retina into the plasma, and so could activate neutrophils migration (Parmar et al., 2018). In this sense, and as we mentioned above, some authors have found abnormally elevated levels of LCN2 and neutrophils activated in the plasma of AMD patients (Fig. 2) (Chen et al., 2020; Ghosh et al., 2019; Parmar et al., 2018). In agreement with this last result, Ilhan et al. have suggested that AMD patients (with both non-vascular and vascular disease) present a high neutrophil-to-lymphocyte ratio (NLR) compared to controls (Fig. 2) (Ilhan et al., 2015). However, other authors have only found an increased NLR value in neovascular AMD patients (Kurtul and Ozer, 2016; Niazi et al., 2019). Consequently, these authors suggest that the LCN2 level and NLR value could be biomarkers of AMD. Rhezar-Dreindl and colleagues have also suggested that the level of LCN2 in the aqueous humor could be a biomarker for monitoring the treatment of persistent/recurrent neovascular AMD patients (Fig. 2) (Rezar-Dreindl et al., 2016).

Regarding the therapeutic target role of LCN2, Ghosh et al. have demonstrated that the intravitreal injection of a serine/threonine-protein kinase AKT2 inhibitor in early AMD-like phenotype mice can mitigate drusen formation, inflammation, neutrophils infiltration, and activation of retinal glial cells (Ghosh et al., 2019). Therefore, targeting AKT2 an upstream regulator of the retinal LCN2 mRNA expression could be a feasible strategy for treating early AMD (Fig. 2) (Ghosh et al., 2019, 2017).

Other potential AMD therapeutic agents are ligands of neutrophil receptors like lipoxin A4, which has been suggested with a limited experimental basis (Bazan, 2009, 2010; Das, 2012), and purinergic blocking agents (Fig. 2) (Fletcher et al., 2019; Notomi et al., 2013).

Fletcher et al. have recently suggested targeting the $P2X_7$ receptor for treating AMD (Fletcher et al., 2019). Indeed, the blockage of the $P2X_7$ receptor delay photoreceptors' death in experimental-late AMD (Notomi et al., 2013). Under inflammatory stress, immune cells like neutrophils release ATP that activates $P2X_7$-induced cell death (Dosch et al., 2018). Besides, ATP can activate the neutrophil $P2X_7$ receptor modulating functions like the secretion of pro-inflammatory mediators, and NET formation (Dosch et al., 2018; Pelegrin and Surprenant, 2009; Sofoluwe et al., 2019; Wang and Chen, 2018). These data explain the potential neuroprotective effect of $P2X_7$ receptor-blocking agents (e.g., antagonists and silencers) in AMD. However, further preclinical, and clinical works are necessary to prove the efficacy of these agents in AMD, including the newer blood-brain barrier-permeable $P2X_7$ antagonists developed (Fletcher et al., 2019).

2.4. Diabetic retinopathy

Diabetic retinopathy (DR) is a common retinal microvascular complication of diabetes (Cheung et al., 2010). Chronic hyperglycemia associated with diabetes leads to oxidative and inflammatory cellular damage that alters the inner blood-retinal barrier (iBRB), allowing plasma leakage, which causes injury to retinal cells (Chiu and Taylor, 2011; Crooke et al., 2017; Eshaq et al., 2017; Yang et al., 2020). Moreover, in the disease's late stage called proliferative DR (PDR), these damages evoke the growth of vessels that invade the vitreous humor, causing blindness (Cheung et al., 2010; Eshaq et al., 2017). Indeed, and according to a 2021 Vision Loss Expert Group report, DR is one of the most common causes of blindness worldwide (GBD, 2019 Blindness and Vision Impairment Collaborators and VLEG, 2021).

The iBRB impairment can also allow neutrophils migration to the retina generating critical events in the pathogenesis of the DR, such as retinal leukostasis and inflammatory response (Wang et al., 2018). In this sense, Wang et al. have demonstrated that the retina of diabetic rats presents an abnormal proportion of neutrophils and overexpresses pro-inflammatory cytokines (e.g., IL-6, IL-8, and IL-1β) (Fig. 2) (Wang et al., 2018). These cytokines, together with hyperglycemia, promote the NETosis process (Delgado-Rizo et al., 2017; Wang et al., 2018). Consequently, various authors have found high levels of NETs and their components (e.g., eDNA, NE, MPO, and LCN2) in the retina of diabetic rats and the vitreous fluid/blood of DR patients (Fig. 2) (Chung et al., 2016; Park et al., 2016; Wang et al., 2018). Furthermore, blood arginine (a precursor of citrulline) and citrulline are abnormally high in DR patients. Nevertheless, the level of systemic/retinal citrullination in these patients remains unknown (Peters et al., 2021).

Regarding the NE enzyme, Liu and coworkers have confirmed the role of this protease in the vascular damage generated in the disease (Liu et al., 2019). Likewise, and given that there is a significant correlation between vitreous LCN2 and VEGF levels in DR patients, LCN2 could also contribute to vascular impairment (Wang et al., 2020). Indeed, some authors have reported that LCN2 can induce the production of hypoxia inducible factor-1α (HIF-1α) and the downstream gene, VEGF, to stimulate angiogenesis (Hu et al., 2018).

The MMP-9/LCN2 complex could also be involved in DR vascular damage, as some authors have suggested that this occurs in other eye vascular diseases (Ghosh et al., 2020; Shen et al., 2016). This complex avoids the inactivation of MMP-9, which might increase the retinal vascular permeability and neovascularization (Kowluru et al., 2012). Additionally, LCN2 can enhance the binding of advanced glycation end-products (AGEs) to their receptors (RAGEs), amplifying diabetic vascular and inflammatory damage (Chiu and Taylor, 2011; Chung et al., 2016; Ciudin et al., 2010). Conversely, these AGEs increase the expression of LCN2 by vascular cells (Chung et al., 2013). Therefore, LCN2 secreted under AGEs- and inflammatory stimuli by retinal cells (vascular, ganglion, glial and recruited-neutrophil cells) could also modulate, via binding to its receptors on these cells, diabetic retinal neuroinflammation as seems to occur in AMD (Chung et al., 2013; Gerhardinger et al., 2005; Ghosh et al., 2020; Parmar et al., 2018).

These results suggest that the neutrophils and NET components, such as LCN2, also play a critical role in DR pathogenesis and could be biomarkers and therapeutic targets for this disease. In this sense, some authors have found elevated NRL values in DR patients mainly in PDR ones compared to controls and suggested its potential as a diagnostic marker of DR severity (Fig. 2) (Ulu et al., 2013; Yue et al., 2015). Moreover, some preclinical studies have suggested the therapeutic potential of indirect NETosis-inhibitors against DR (Fig. 2). In this context, Purvis and coworkers have demonstrated the protective effects of annexin-1 against microvascular complications in an animal model of type 1 diabetes (Purvis et al., 2018). The treatment of diabetic mice with human recombinant annexin-1 inhibits mitogen-activated protein kinases (MAPKs), which are activated in hyperglycemic condition, attenuating the cardiac and renal dysfunction of these animals (Purvis et al., 2018). Given that MAPKs also mediate DR, annexin-1 could have a therapeutic effect against this microvascular complication (Purvis et al., 2018). Likewise, some authors have proposed the therapeutic potential of lipoxin A4 against diabetes-associated vascular complications (Brennan et al., 2018; Das, 2012, 2013). Indeed, Brennan et al. have demonstrated that lipoxin A4 and its analog benzo-lipoxin A4 protect against vascular inflammatory damage in aortic tissue of diabetic rats and human carotid atherosclerotic plaques (*ex-vivo*) (Brennan et al., 2018). These lipidic mediators reduce the production of pro-inflammatory cytokines and VEGF-induced angiogenesis (Das, 2012). Therefore, lipoxin A4 and its analogs could protect against PDR (Das, 2012, 2013).

Other regulators of neutrophil functions with therapeutic potential in DR are the ligands of purinergic receptors (Fig. 2) (Clapp et al., 2019; Liou et al., 2011; Mancini et al., 2018; Sugiyama, 2014; Vindeirinho et al., 2016). The continuous ATP released by retinal cells like ganglion, glial and vascular cells and infiltrated neutrophil cells during diabetes activates the purinergic receptors expressed by these cells (Costa et al., 2009; Mancini et al., 2018). So, the activation of retinal $P2X_7$ receptors can evoke vascular cell death and regulate the secretion of pro-inflammatory mediators (Clapp et al., 2019; Sugiyama et al., 2005). Indeed, the blockage of $P2X_7$ receptors reverses increased vascular

permeability, VEGF accumulation, and IL-6 expression in diabetic rat retinas (Clapp et al., 2019). Similarly, Mancini et al. have proved that the inhibition of the $P2X_2$ receptor decreases retinal cell death in diabetic rats (Mancini et al., 2018). In contrast, the effect of adenosine receptor ligands in the retina during diabetes is controversial. For example, adenosine A2 receptor (ADORA2A) agonists could prevent or promote vascular damage depending on the cell type and the experimental approach (Barletta et al., 2012; Charles, 2014; Liou et al., 2011; Vindeirinho et al., 2016). These agonists can decrease neutrophil adherence to endothelial cells (prevent vascular damage) and increase the production of retinal VEGF or glucose transport into retinal vasculature (promote vascular damage) (Barletta et al., 2012; Takagi et al., 1998, 1996).

On the other hand, inhibitors of the adenosine reuptake (adenosine transporters) are under investigation as a therapeutic alternative of adenosine and its analogs against DR (Liou et al., 2011). These inhibitors have been successfully probed in experimental diabetes, decreasing microglial activation and retinal vasculature damage (De la Cruz et al., 1997; Liou et al., 2008).

The translation of all these preclinical data into clinical practice will require a better characterization of the DR underlying molecular mechanisms mediated by all these components of the adenosinergic system.

3. Conclusions and future directions

Age-related eye diseases, including dry eye, glaucoma, age-related macular degeneration, and diabetic retinopathy, represent a major global health issue based on their increasing prevalence and disabling action, with an enormous personal and economic consequences (GBD, 2019 Blindness and Vision Impairment Collaborators and VLEG, 2021; Uchino and Schaumberg, 2013). In this sense, The Lancet Global Health Commission on Global Eye Health argues that to achieve eye health (and thus facilitates the UN Sustainable Development Goals) is necessary to understand the mechanisms underlying age-related eye diseases (Burton et al., 2021).

One of these mechanisms shared with other age-related diseases seems to be the impairment of neutrophil extracellular traps formation/degradation processes. Indeed, age-related eye disease patients present local recruitment of neutrophils and overexpression of neutrophil extracellular trap components at local and systemic levels (An et al., 2019; Arafat et al., 2017; Aragona et al., 2006; Chau et al., 2007; Chen et al., 2020; Chung et al., 2016; Ghosh et al., 2019, 2017; Guo et al., 2011; Kaeslin et al., 2016; Park et al., 2016; Reyes et al., 2018; Sonawane et al., 2012; Tibrewal et al., 2013; Ueno et al., 2018; Wang et al., 2018).

These data suggest the potential role of neutrophils and their constituents as pathologic mediators, biomarkers, and therapeutic targets of AREDs. In this context, future studies should explore the validity of AREDs biomarkers linked to neutrophils at local and particularly at ocular surface level. Tear film and ocular surface cells represent a power source of biomarkers of ocular and systemic diseases like neurodegenerative disorders (Hagan et al., 2016; Roda et al., 2020). Furthermore, the techniques employed to obtain tear and ocular surface samples are less invasive than blood/intraocular sampling methods. So, it will be interesting to determine the value of the blood indicator of systemic inflammation and poor disease prognosis NLH at the tear level of AREDs patients (including age-related retinal diseases ones). Likewise, and given the pivotal role of LCN2 as a neuroinflammatory and vascular damage modulator, the measure of this NET component in the ocular surface of age-related retinal diseases patients could supply quite beneficial for the diagnostic of these diseases. Indeed, several authors have already proposed the LCN2 diagnostic use (and therapeutic potential of targeting LCN2) for other neurodegenerative disorders (e.g., Alzheimer's, Parkinson's, multiple sclerosis, and Huntington's diseases) (reviewed in (Suk, 2016)).

Regarding the therapeutic potential of targeting the NET components in AREDs, the use of DNase inhibitors and pooled ocular surface immunoglobulin (IgG)-eye drops, which neutralize ACPAs effects, has been particularly successful in DED patients (Kwon et al., 2020; Mun et al., 2019). In this context, Chirivi et al. have proposed as drug candidates to treat NET-mediated inflammatory diseases the so-called therapeutic ACPAs (ACPAs that selectively bind to citrullinated histones 2A and 4) (Chirivi et al., 2021). These therapeutic ACPAs diminish NET release and promote NET clearance *in vivo* (Chirivi et al., 2021). These results could open new therapeutic avenues to combat AREDs. An upstream modulator of LCN2 mRNA has also provided an encouraging result in experimental AMD (Ghosh et al., 2019, 2017). These results should inspire future research concerning the employ of NET components-blocking agents like DNase inhibitors and LCN2 modulators against age-related eye diseases.

Other potential and emerging AREDs therapeutic agents are ligands of neutrophil receptors like pro-resolution mediators and purinergic ligands, which neutralize neutrophils recruitment avoiding local NET components accumulation (Clapp et al., 2019; Das, 2013; Li et al., 2018; Liou et al., 2011; Mancini et al., 2018; Purvis et al., 2018; Romano et al., 2020; Shinozaki et al., 2017; Tibrewal et al., 2014). Future research should confirm these initial (preclinical) findings enabling their translation into clinical practice.

Finally, we also believe that combination therapy approaches including at least one of the above-commented agents targeting neutrophils or NET components are the unique choice to achieve long-term health benefits, given the complexity of AREDs. Consequently, we consider that age-related eye diseases future research should also explore these multi-target drug cocktails.

Author Contributions

Irene Martínez-Alberquilla: Investigation and Writing – original draft. **Xavier Gasull:** Writing – review & editing. **Patricia Pérez-Luna**: Investigation. **Rubén Seco-Mera:** Investigation. **Javier Ruiz-Alcocer:** Investigation, Conceptualization. **Almudena Crooke:** Conceptualization, Supervision, and Writing – original draft.

Declaration of Competing Interest

The authors report no declarations of interest.

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Presbyopia: An outstanding and global opportunity for early detection of pre-frailty and frailty states

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Introduction

According to the World Population Prospects 2022 of the United Nations, the world's population will rise over the next decades (e.g., by 6.3 and 21% from 2022 to 2030 and 2050, respectively) (1). Likewise, the population of older persons is increasing (1). Indeed, the United Nations expects that the share of the global population aged 65 years and above will rise by 6% between 2022 and 2050 (1). Consequently, age-related diseases, including eye ones, are expected to be very prevalent (2–4). For this reason, population aging is a crucial demographic issue with a growing global impact on all socioeconomic areas (4). This fact has provoked different global initiatives like the one declared by the United Nations in 2020, called the Decade of Healthy Aging 2021–2030, whose aim is to promote and maintain the wellbeing of older adults (5). In this context, the Lancet Global Health Commission on Global Eye Health argues that eye health should be part of such strategies focused on achieving universal healthy aging (6). Indeed, vision impairment and blindness affect multiple functional domains (physical, cognitive, psychological, social) and overall quality of life and wellbeing (6). Now, 510 million people have impaired vision, and 43.3 million are blind (7). Among these people, older adults present with a moderate/severe vision impairment and blindness prevalence of 112 cases and 18.5 cases per 1,000 people, respectively (7). Age-related eye diseases (e.g., cataracts, glaucoma, age-related macular degeneration, and diabetic retinopathy) are the leading global causes of visual impairment and blindness (6, 8). Hence, as the population ages, an increase in those numbers is expected, and by 2050 866 million and 61.0 million people will have moderate/severe vision impairment and be blind, respectively (7). Consequently, this global eye health initiative urges, like healthy aging initiatives, an in-depth understanding of the aging process and its associated diseases for preventing or delaying age-related eye conditions (6, 9).

Age-related eye changes: Presbyopia

The aging process involves a progressive decline of all organ-specific functions, including eye ones. Like the rest of the body, the eye undergoes age-triggered changes that alter its structures, impairing its physiological functions [(10) review eye changes critical for the onset of age-related eye diseases]. One of these impaired functions is the accommodation process. This process, which allows focusing on near objects, occurs by the harmonized action of the ciliary muscle and the zonule fibers which hold the lens in place. Aging triggers changes in these structures involved in accommodation, leading to the gradual inability of the eye to focus (11, 12). This physiological event called presbyopia starts to express itself at around 40 years and affects 100% of the population by age 50 (13, 14). The tell-tale symptom of presbyopia is blurred vision while reading, sewing, using a mobile phone, tablet, computer, or doing anything that requires intermediate and near vision (5). Furthermore, presbyopia may present with eyestrain and headaches after reading or doing close-up work (5). Therefore, it negatively impacts the individuals' quality of life, urging them to seek a solution from an eye specialist (15–17).

Frailty and eye

Frailty is an age-related syndrome that implies changes at all physiological levels, leading to a state of vulnerability, which could facilitate age-related disease onsets (18). A recent systematic review has shown that the overall prevalence of frailty and pre-frailty among individuals aged 50 years and older varies between 12–24% and 46–49%, respectively (19). Likewise, another systematic review has revealed that the incidence of frailty and pre-frailty among older adults is 43.4 and 150.6 new cases per 1,000 person-years, respectively (20).

Several recent studies have identified pre-frailty/frailty signs in middle-aged adults (40–50 years old) (21–23). Even more notable is that this frailty/pre-frailty condition is associated with multimorbidity and mortality in UK older/middle-aged participants of a prospective analysis (22). So, early detection of this condition could allow rapid implantation of measures that prevent or delay these poor health outcomes (22). Unfortunately, its multiple signs and symptoms (often non-specific) and the limited knowledge of its underlying molecular mechanisms (mainly in middle-aged adults) have hindered its early diagnosis (18).

The World Health Organization (WHO) has introduced the concept of intrinsic capacity (IC) (i.e., the combination of all the individual's physical and mental capacities) as a crucial component of healthy aging (24). It has also provided recommendations and tools to manage IC decline at the community level and primary care level, assuring integrated care for older adults (ICOPE) (25, 26).

According to the WHO ICOPE guidelines, vision is a critical component of IC (26). A simple eye chart permits the measurement of visual capacity, and distance acuity worse than 6/18 implies moderate vision impairment that needs further diagnostic assessment (26).

In one US study with 2,705 older adults, individuals with near vision impairment were more likely to be pre-frail and frail than those without visual loss (27). This result suggests an association between vision impairment, which promotes IC decline, and frailty (27). In this sense, some prospective studies have found that IC decline overlaps with frailty syndrome and can predict poor health outcomes in older adults (28–30).

Another UK prospective study with 493,737 middle-aged adults and older adults showed that individuals with glaucoma had a high prevalence of pre-frailty and frailty conditions (41.2 and 5%, respectively) (22). Equally, Wang et al. have found in a prospective China population-based study that this disease is associated with 10-year mortality (31).

The previously mentioned study by Hanlon et al. of middle-aged and older adults also revealed that patients with diabetes presented higher pre-frailty and frailty prevalence (54.8 and 13%, respectively) than glaucomatous ones (22). Besides, a systematic review has proved an association between diabetic retinopathy (DR, a major microvascular diabetes complication) and poor psychosocial functioning, affecting the quality of life of these patients (8, 32, 33). Likewise, a retrospective cohort study with 477 participants found that both frailty and diabetic microvascular complications can predict adverse clinical outcomes (e.g., emergency hospitalizations, institutionalization in a long-term care facility, falls, fractures, and death) in diabetic older adults (34).

Although the prevalence of pre-frailty/frailty in individuals with age-related macular degeneration (AMD) is yet unknown, Zhu et al. have suggested in a prospective study that late AMD is a biomarker of frailty syndrome (35). These authors argued that the poor survival observed in late AMD participants could be due to age- and frailty-related systemic comorbidities that coexist and share underlying molecular mechanisms with AMD (35). Moreover, several prospective studies have demonstrated that patients with AMD present a higher risk of falls and fear of falling, which leads to a decreased quality of life and disability (36–38). This fear of falling also has been observed in glaucomatous patients using the same validated questionnaire (the University of Illinois at Chicago Fear of Falling Questionnaire) (39). Some systematic reviews have also demonstrated an association between the fear of falling and poor quality of life with frailty (40, 41). Therefore, all these data seem to connect AMD and frailty.

A prospective study performed with age-related cataract patients found that they have poor survival rates, suggesting that cataracts, the most important cause of visual impairment and blindness, are also a biomarker of frailty (42). This study confirmed a previous cohort study that had shown

the association between age-related cataracts and some measures of frailty independent of visual acuity and systemic comorbidities (43).

Villani et al. have built an ocular surface frailty index (OSFI) and tested *via* a longitudinal study its capacity to identify frail-ocular surfaces among patients who underwent cataract surgery (44). Consequently, these authors propose OSFI as a tool to predict patients with a high risk of post-surgical development of dry eye disease (DED) (44). This disease is also an age-related condition of the ocular surface that represents a growing problem with a substantial negative impact on the quality of life and global economy (45, 46).

To support this subsection, we searched in PubMed for the combination of the words: "frailty" and "eye" or one of the four age-related eye diseases leading causes of visual impairment/blindness: "cataracts", "glaucoma", "age-related macular degeneration", and "diabetic retinopathy". As we only aimed to summarize knowledge concerning this subsection's topic, among all articles found, we selected those most recent and focused on our point of view.

Eye as a source of diagnostic biomarkers

The eye and especially the tear film have become, in recent years, the target for researchers of being an outstanding source of biomarkers for the diagnosis of both ocular and systemic diseases such as dry eye, Sjogren's syndrome, keratoconus, cancer, and COVID-19 (47–52). The main reason for this is that tears are the most accessible corporal fluid, and collecting them is easier, faster, and less invasive than the collection methods of other fluids (53).

Tear film covers the external ocular surface and consists of an inner mucous/aqueous and an external lipid phase, presenting a great diversity of macromolecules that undergo measurable changes in pathological conditions (54–58). Among these conditions are age-related diseases, including eye diseases. So, tears have provided several potential biomarkers for cataracts, glaucoma, AMD, DR, Alzheimer's, and Parkinson's diseases (57, 59–63). Conversely, the presence of specific frailty biomarkers in tears is unknown.

Discussion

Age is a driving factor for frailty and age-related diseases, sharing underlying molecular mechanisms (64–66). According to the population aging and life expectancy prospects, these conditions, including eye-related ones, will be very prevalent (1, 2, 64). Age-related eye diseases are the world leading causes of visual impairment and blindness (6, 8). Hence, as the population ages, a growing number of visually impaired and blind people is expected, which will have an enormous humanistic and economic impact (8). These visual problems decrease the IC and quality of life of those affected by it and are associated with frailty syndrome (6–8, 27). Moreover, age-related eye diseases coexist with pre-frailty/frailty syndrome and are potential biomarkers of frailty and predictors of poor health outcomes (8, 22, 31, 34, 35, 42). These data reflect the crucial role of visual performance in achieving healthy aging (6). In this context, future studies should explore the validity of including new visual function-related tests in primary care for the integrated attention of older adults (6, 37, 39–41, 67). Indeed, some vision experts claim to perform the contrast sensitivity test to evaluate the fear of falling (a marker of poor quality of life, disability, and frailty) because it is a better predictor of this fear than the visual acuity test (37). Moreover, a recent cross-sectional study has found that poor contrast sensitivity is associated with frailty (68). Likewise, the older adults' health programs could include questionnaires to measure fear of falling and quality of life previously validated in patients with age-related eye diseases (37, 39, 67).

The pivotal role of visual performance in achieving healthy aging also urges research in the diagnosis and treatment of age-related eye disorders fields to implement new global preventive and therapeutic strategies against those diseases (6).

Frailty syndrome, a geriatrician's high-priority theme, has become an emerging target of gerontologists. They have found that this disorder that predisposes a person to age-related disease onsets is present in older and middle-aged adults and is associated with mortality, particularly in individuals with multi-morbidity (18, 21–23, 69). Given that a rapid intervention can reverse the condition, thus preventing its poor health outcome, gerontologists recommend screening frailty biomarkers in middle-aged adults (from the fourth decade of life onwards) (21–23, 69).

In this life period, presbyopia can also occur. This physiological process gradually reduces the ability of the eye to focus at different distances, impacting individuals without and with refractive errors (e.g., myopia, hyperopia, or astigmatism) who start to feel presbyopia symptoms from 40–50 years (12, 14). Because of these presbyopic symptoms, the entire population of middle-aged adults will visit eye care professionals seeking a solution. Probably, no other biomedical professionals attend to the whole population of middle-aged adults. This fact is remarkable because, as we have commented above, screening frailty biomarkers in middle-aged adults is critical for timely interventions to prevent age-related diseases and mortality.

Some data support the concept of age-related eye diseases as biomarkers of frailty phenotype and predictors of poor health outcomes (8, 22, 27, 31, 34, 35, 42, 67). Equally, data back the concept of the eye and its tear as a source of diagnostic biomarkers of ocular and systemic diseases, including age-related ones (57, 59–63). Thus, it would not be surprising that tears would contain frailty biomarkers. As any eye practitioner can easily

collect tears, screening for frailty biomarkers from tears of presbyopic subjects may represent an outstanding opportunity for early detection of pre-frailty and frailty states, allowing timely intervention and thus preventing poor clinical outcomes.

Molecular mechanisms of frailty could also arise at the eye level, as occur with aging and age-related diseases (65). Indeed, a systematic review has revealed recently that frailty mechanisms occur in oral tissues (70). In this sense, the prospective study of Villani et al. has suggested the existence of frailty underlying mechanisms at the ocular surface of individuals who undergo cataract surgery (44). Thus, a future screening of frailty biomarkers from tears of presbyopic subjects could be a simple method of studying possible ocular surface frailty mechanisms and how they could link to the processes that occur in the rest of the eye and body. The understanding of these mechanisms could provide new biomarkers, helping delay age-related diseases onsets, including eye ones.

In summary, this article aims to show that theoretically, it is possible to perform a simple and large-scale frailty screening of middle-aged adults' tears, taking advantage of the unavoidable visit of presbyopic individuals to eye care professionals looking for a solution to their symptoms. The previous search for frailty biomarkers taken from tears of presbyopic people would allow this screening and thus timely interventions, delaying age-related diseases onsets and mortality.

We have confidence in the value of the tears of presbyopic people as an easy means to identify frail/pre-frail individuals, validate frailty biomarkers candidates, and study local frailty molecular mechanisms, which will provide new biomarkers.

Author contributions

AC, DM-C, and JR-A contributed to the research conception. AC, JR-A, and IM-A contributed to the literature review. AC and JR-A contributed to the manuscript writing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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