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New Advances and Perspectives in Ophthalmology

Progress and Modern Challenges

Edited by
Livio Vitiello

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Guest Editor

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About the Editor

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Journal of
Personalized Medicine

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Editorial

New Advances and Perspectives in Ophthalmology: Progress and Modern Challenges Toward Personalized Eye Care

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1. Recent Developments in Ophthalmology

Over the past two decades, ophthalmology has been reshaped by the convergence of high-resolution multimodal imaging, pharmacological innovation and the broader paradigm of personalized medicine. Swept-source optical coherence tomography (OCT), OCT angiography, ultra-widefield retinal imaging and high-frequency ocular ultrasound now enable in vivo, micrometer-scale phenotyping of virtually every ocular structure [1], while artificial intelligence (AI) and deep learning turn these imaging outputs into quantitative biomarkers suitable for automated risk stratification [2,3]. In parallel, the therapeutic armamentarium has expanded from intravitreal anti-VEGF biologics—now available in longer-acting formulations and individualized treat-and-extend regimens [4]—to gene and cell therapies for inherited retinal dystrophies [5,6], and to increasingly refined cataract, refractive and oculoplastic platforms. Underlying these advances is a conceptual shift: ophthalmic diseases are no longer treated as homogeneous entities, but rather as heterogeneous clusters of phenotypes, each with their own risk profiles and optimal management.

2. Knowledge Gaps Addressed by This Special Issue

Despite this progress, several important gaps remain: real-world long-term data on individualized anti-VEGF regimens are still limited; ROP screening is inequitably distributed and logistically demanding; AI-based screening tools often lack prospective validation and safe-failure mechanisms [7]; IOL calculation remains problematic in post-refractive eyes [8]; predictors of surgical success in strabismus are poorly standardized; postural and dynamic components of intraocular pressure (IOP) are rarely integrated into routine glaucoma risk assessment; ocular imaging biomarkers of systemic disease are still being defined [9]; subclinical dysfunction in "inactive" orbitopathy is underdiagnosed; and the nutritional management of age-related macular degeneration (AMD) has evolved beyond the original AREDS framework without a consensus on individualized protocols. The sixteen contributions collected in this Special Issue directly address these gaps and sketch a coherent roadmap toward more personalized ophthalmic care.

3. Key Findings and Contributions

3.1. Retinal Vascular Disease and Anti-VEGF Personalization

Diabetic retinopathy (DR) remains a paradigmatic setting for personalized medicine. Zhou et al. (contribution 1) report 1-year outcomes of the VOYAGE extension study: in patients who had completed the PANORAMA trial and received 2 mg intravitreal aflibercept every 16 weeks as needed, less frequent dosing between PANORAMA exit and VOYAGE enrollment was associated with a measurable deterioration of the Diabetic Retinopathy Severity Scale score, best-corrected visual acuity and central subfield thickness,

which subsequently stabilized or improved once regular, individualized treatment resumed. These findings are real-world evidence that anti-VEGF schedules must be tailored to ongoing disease activity. Ivanescu et al. (contribution 2) profiled 105 patients with type 1 or type 2 diabetes (77 with diabetic macular edema) in Western Romania, describing a high-risk cohort with median age 65 years, 15-year mean disease duration, HbA1c 7.5% and universal comorbidities (hypertension 100%, dyslipidemia 62.3%)—reinforcing the need for screening pathways integrated with systemic risk stratification.

3.2. Retinopathy of Prematurity: From Universal Screening to Tailored Pathways

Two complementary studies address retinopathy of prematurity. Christodoulou et al. (contribution 3) evaluated incidence, treatment patterns and risk factors for type 1 ROP in a national tertiary NICU in Cyprus, translating local epidemiology into a personalized screening-to-therapy pathway. Al-Abaiji et al. (contribution 4) demonstrated the feasibility of telescreening in Denmark using the ICON GO® widefield camera operated by a non-physician healthcare professional: across 415 sessions in 165 neonates, on-site/off-site agreement was almost perfect for overall outcome ($\kappa = 0.82$) and substantial for stage and disease ($\kappa = 0.69$), with treatment needed in 3.6% of children. These findings provide evidence that delegated, tele-supported screening is safe, scalable, and a concrete step toward equitable neonatal care.

3.3. Artificial Intelligence and Glaucoma Screening

Hitzl et al. (contribution 5) leveraged a longitudinal dataset of 6889 subjects, with 585 completing an average 11.1-year follow-up period, to train two neural-network models equipped with a "reject option" that allows the algorithm to withhold a prediction in ambiguous cases. The clinical and economic rationale is to identify, already at baseline, the large proportion of screened individuals who remain free of open-angle glaucoma for up to 10 years and can therefore safely be excluded from intensive monitoring—a concrete translation of precision screening into everyday practice.

3.4. Precision in Refractive, Cataract and Strabismus Surgery

Tailored surgical planning is the focus of two studies. Lin et al. (contribution 6) compared the calibration accuracy of the Symfony extended depth-of-focus (EDOF) intraocular lens in eyes with and without previous LASIK, combining optical biometry with the SRK/T formula in non-LASIK eyes and Haigis-L in post-LASIK eyes—a clinically actionable framework for IOL power selection in complex refractive histories. Lino, de Aguiar and Cunha (contribution 7) retrospectively analyzed 258 children (mean age 11.2 years) with basic-type intermittent exotropia or divergence excess who underwent bilateral lateral rectus recession, identifying pre-operative predictors of post-operative alignment stability and refining the individualized surgical dosing needed to improve on the historical $\sim$75% success rate.

3.5. Intraocular Pressure, Biometrics and Glaucoma Biomechanics

De Bernardo et al. (contribution 8) measured IOP across sitting, supine, prone and standing postures in 124 eyes of 62 healthy subjects: the prone–supine differential was the largest (+2.24 mmHg; $p < 0.001$), followed by supine–sitting (+1.30 mmHg), while standing produced a significant IOP decrease relative to supine (-1.43 mmHg). Such postural profiles, which a single in-office measurement cannot capture, argue for a more individualized interpretation of IOP in selected glaucoma suspects. Gioia et al. (contribution 9) correlated the corrected axial length—a refinement addressing the systematic biometric error of cataractous eyes—with choroidal thickness, refining the quantitative framework for myopic and degenerative risk stratification.

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3.6. Imaging Biomarkers and the Ocular–Systemic Interface

Three contributions use imaging biomarkers as windows onto systemic disease. Isleyen et al. (contribution 10) applied swept-source OCT to 67 patients with vasovagal syncope versus 61 controls, demonstrating a significantly thicker choroid in affected subjects without changes in peripapillary retinal nerve fiber layer (RNFL), showing that the choroidal vasculature is sensitive to autonomic dysregulation. Lixi et al. (contribution 11) reviewed the spectrum of ocular manifestations of patent foramen ovale, from transient visual disturbances and migraine with aura to oculomotor deficits and rare endogenous endophthalmitis from paradoxical embolism, reinforcing the need for a multidisciplinary ophthalmology–cardiology–neurology pathway. In 67 patients with inactive Graves' orbitopathy, Lixi, Giannaccare and co-authors (contribution 12) combined orbital ultrasound with the Red Filter Test and detected latent diplopia in 49.25% of subjects despite no reported double vision—a strong case for sensitivity-based reassessment in a disease too often labeled "burnt out".

3.7. Genomics and Precision Medicine in Inherited Retinal Disease

Two contributions address the genomic frontier of personalized ophthalmology. Dhivagaran et al. (contribution 13) provide a comprehensive review of precision medicine in inherited retinal diseases (IRDs), tracing the evolution from clinical phenotyping to next-generation sequencing (NGS)-based diagnostics and mutation-specific therapies. The review details how NGS has transformed the diagnostic landscape by enabling precise identification of causative variants across the more than 300 genes implicated in IRDs, and examines the patient-selection protocols—integrating genetic confirmation, structural retinal evaluation and genetic counseling—that underpin targeted interventions such as voretigene neparvovec (Luxturna) for RPE65-related disease. The authors also map the remaining barriers to broader adoption: high costs, uneven access to specialist services, and the interpretive complexity of variants of uncertain significance. Bakhamees et al. (contribution 14) complement this perspective with a cross-sectional survey of 115 ophthalmologists across Saudi Arabia, assessing knowledge, attitudes and practices toward genomic medicine. While 99% correctly recognized autosomal recessive inheritance and 90% endorsed the clinical value of genetic testing, confidence in practical tasks such as test selection and pre-test counselling was low (mean 4.7/10), and only 9% answered DNA base-pairing questions correctly. Taken together, these two contributions delineate a critical gap between the rapid maturation of genomic tools and the readiness of the clinical workforce to deploy them—and underscore the urgency of integrating genetics into ophthalmology training programs.

3.8. Diagnostic Imaging and Tailored Medical Therapy

Two narrative reviews complete this Special Issue. Coppola et al. (contribution 15) synthesized the literature on ocular ultrasound for optic neuropathies, emphasizing its bedside availability and particular value in patients unable to undergo magnetic resonance imaging, while acknowledging the methodological heterogeneity that still characterizes the field [10–12]. D'Angelo et al. (contribution 16) reviewed the evolving evidence on oral supplementation in AMD—the third leading cause of global visual impairment—mapping the interplay between oxidative stress, neuroinflammation and ocular ischemia, and discussing AREDS-based formulations alongside emerging compounds such as astaxanthin and melatonin. Their synthesis supports the move from one-size-fits-all supplementation toward individualized antioxidant strategies.

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4. Future Research Directions

The contributions collected here open several avenues that will shape the next phase of personalized ophthalmology. First, imaging biomarkers—from choroidal thickness and RNFL to corrected axial length and extraocular muscle measurements—need cross-platform standardization, with consensus on acquisition protocols, reference ranges and reproducibility thresholds, before they can reliably guide individual decisions. Second, machine learning models should undergo prospective, multicenter validation in ethnically and demographically heterogeneous populations, ideally with confidence-aware outputs and human-in-the-loop oversight. Third, the integration of telemedicine, AI and portable imaging must be evaluated not only for diagnostic accuracy but also for cost-effectiveness and equity of access, particularly in vulnerable populations. Fourth, evidence on postural IOP, subclinical ocular motility dysfunction and ocular signs of systemic disease calls for the systematic integration of ophthalmology into cardio-, neuro- and endocrine-ophthalmology pathways. Fifth, pharmacological and nutritional management should move beyond population-average recommendations toward genotype-, phenotype- and biomarker-guided regimens for anti-VEGF therapy in DR/DME, IOL selection in complex refractive histories and antioxidant supplementation in AMD. Finally, patient-reported outcomes and adherence metrics must be prospectively incorporated into personalized protocols, so that success is defined not only by anatomical and functional endpoints but also by what matters most to patients.

5. Concluding Remarks

Collectively, the sixteen contributions in this Special Issue trace a coherent trajectory for contemporary ophthalmology: broader and earlier screening underpinned by telemedicine and AI; finer phenotyping supported by multimodal imaging and advanced biometrics; and more individualized therapeutic choices, from anti-VEGF regimens and IOL calculations to surgical dosing and antioxidant supplementation. The work presented here offers concrete building blocks for a genuinely personalized ophthalmic practice—and a clear agenda for the studies that will follow.

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List of Contributions:

1. Zhou, A.W.; Teagle, G.M.; Baumann, L.M.; Cao, J.A.; Emanuelli, A.; Hu, A.Y.; Berger, A.S.; Major, J.C., Jr.; Lee, S.Y.; Huddleston, S.M.; et al. Intravitreal Aflibercept for the Treatment of Diabetic Retinopathy Among Patients Who Completed PANORAMA: 1-Year Outcomes from the VOYAGE Extension Study. *J. Pers. Med.* **2025**, *15*, 555. https://doi.org/10.3390/jpm15110555.
2. Ivanescu, A.; Popescu, S.; Roman, D.; Dragomir, M.; Timar, R. Diabetic Macular Edema in the Western Part of Romania: Screening to Improve Patient Outcomes. *J. Pers. Med.* **2025**, *15*, 106. https://doi.org/10.3390/jpm15030106.
3. Christodoulou, S.; Herodotou, F.; Quattrocchi, A.; Potamitis, T.; Choleva, V. From Screening to Therapy: A Personalized Approach to ROP in a National NICU Setting. *J. Pers. Med.* **2025**, *15*, 388. https://doi.org/10.3390/jpm15080388.

4. Al-Abaiji, H.A.; Bangsgaard, R.; Kofod, M.; Faber, C.; Larsen, A.-C.; Brost, A.G.; Slidsborg, C.; Klemp, K.; Breindahl, M.; la Cour, M.D.d.; et al. Evaluating the Feasibility of a Telescreening Program for Retinopathy of Prematurity (ROP) in Denmark. *J. Pers. Med.* **2024**, *14*, 1020. https://doi.org/10.3390/jpm14101020.
5. Hitzl, W.; Lenzhofer, M.; Hohensinn, M.; Reitsamer, H.A. Two Machine Learning Models to Economize Glaucoma Screening Programs: An Approach Based on Neural Nets. *J. Pers. Med.* **2025**, *15*, 361. https://doi.org/10.3390/jpm15080361.
6. Lin, I.-H.; Chao, C.-C.; Chang, C.-K. Effect of Prior Laser-Assisted In Situ Keratomileusis on the Calibration Accuracy of Extended Depth of Focus Intraocular Lenses: A Direct Comparative Study. *J. Pers. Med.* **2025**, *15*, 301. https://doi.org/10.3390/jpm15070301.
7. Lino, P.; de Aguiar, P.V.; Cunha, J.P. Success of Strabismus Surgery in Intermittent Exotropia. *J. Pers. Med.* **2025**, *15*, 333. https://doi.org/10.3390/jpm15080333.
8. De Bernardo, M.; Cione, F.; De Pascale, I.; Pagliarulo, S.; Rosa, N. Intraocular Pressure Measurements in Standing, Sitting, Prone, and Supine Positions. *J. Pers. Med.* **2024**, *14*, 826. https://doi.org/10.3390/jpm14080826.
9. Gioia, M.; De Bernardo, M.; Cione, F.; De Luca, M.; Rosa, N. Corrected Axial Length and Choroidal Thickness: A Correlation Analysis for Scientific Purposes. *J. Pers. Med.* **2025**, *15*, 15. https://doi.org/10.3390/jpm15010015.
10. Isleyen, H.B.; Kanar, B.G.; Akcay, G.; Demir, S.; Kanar, H.S.; Yazicioglu, M.V. Evaluation of the Choroidal Thickness and Retinal Nerve Fiber Layer Thickness in Patients with Vasovagal Syncope. *J. Pers. Med.* **2025**, *15*, 259. https://doi.org/10.3390/jpm15060259.
11. Lixi, F.; Cuccu, A.; Giannaccare, G.; Onnis, M.; Timofte Zorila, M.M.; Mariotti, S.; Vacca, R.; Meloni, P.E.; Pisu, M.; Mura, C.; et al. Subclinical Ocular Motility Dysfunction and Extraocular Muscle Changes in Inactive Graves' Orbitopathy. *J. Pers. Med.* **2024**, *14*, 848. https://doi.org/10.3390/jpm14080848.
12. Lixi, F.; Fazzini, L.; Cannas, C.; Montisci, R.; Giannaccare, G. Ocular Manifestations and Complications of Patent Foramen Ovale: A Narrative Review. *J. Pers. Med.* **2024**, *14*, 695. https://doi.org/10.3390/jpm14070695.
13. Dhivagaran, T.; Butt, F.R.; Grover, K.; Devgan, K.; Sachdeva, K.; Dhivagaran, V.; Abid, F.; Tao, B.K.; Balas, M.; Dimopoulos, I.; et al. Precision Medicine in Inherited Retinal Disease: Advances, Challenges, and Future Directions. *J. Pers. Med.* **2026**, *16*, 292. https://doi.org/10.3390/jpm16060292.
14. Bakhamees, W.; Alsafran, H.; ALBalawi, H.B.; Alali, N.M.; Alotaibi, Y.A.; Magliyah, M.S. Genomic Medicine Among Ophthalmologists: Knowledge, Current Practice, and Barriers. *J. Pers. Med.* **2026**, *16*, 267. https://doi.org/10.3390/jpm16050267.
15. Coppola, A.; Abbinante, G.; De Pascale, I.; Gagliardi, V.; Salerno, G.; Pellegrino, A.; Vitiello, L. Ocular Ultrasound in the Diagnosis of Optic Neuropathies: A Review of the Literature. *J. Pers. Med.* **2024**, *14*, 949. https://doi.org/10.3390/jpm14090949.
16. D'Angelo, A.; Vitiello, L.; Gagliardi, V.; Salerno, G.; De Pascale, I.; Coppola, A.; Abbinante, G.; Pellegrino, A.; Giannaccare, G. The Role of Oral Supplementation for the Management of Age-Related Macular Degeneration: A Narrative Review. *J. Pers. Med.* **2024**, *14*, 653. https://doi.org/10.3390/jpm14060653.

References

1. Wong, Q.Y.; Sim, R.; Ang, M. Anterior Segment Optical Coherence Tomography with Angiography for the Cornea and Ocular Surface. *J. Clin. Med.* **2026**, *15*, 2402. [CrossRef] [PubMed]
2. Parravano, M.; Cennamo, G.; Di Antonio, L.; Grassi, M.O.; Lupidi, M.; Rispoli, M.; Savastano, M.C.; Veritti, D.; Vujosevic, S. Multimodal imaging in diabetic retinopathy and macular edema: An update about biomarkers. *Surv. Ophthalmol.* **2024**, *69*, 893–904. [CrossRef] [PubMed]
3. Rispoli, M.; Cennamo, G.; Antonio, L.D.; Lupidi, M.; Parravano, M.; Pellegrini, M.; Veritti, D.; Vujosevic, S.; Savastano, M.C. Practical guidance for imaging biomarkers in exudative age-related macular degeneration. *Surv. Ophthalmol.* **2023**, *68*, 615–627. [CrossRef] [PubMed]

4. Menna, F.; De Luca, L.; Meduri, A.; Baldascino, A.; Lupo, S.; Vingolo, E.M. New Clinical Trials and Therapeutic Advances with Faricimab and Aflibercept 8 mg in Neovascular Age-Related Macular Degeneration: A Durability-Oriented Comparative Perspective. *Biomedicines* **2026**, *14*, 773. [CrossRef] [PubMed]
5. Ekemiri, K.; Daniel, E.; Seemongal-Dass, R.; Ekemiri, C.; Victor, V.; Amobi, M.; Adebo, O.; Tekalign, T. Advanced therapeutic approaches for inherited retinal diseases: An umbrella review. *BMJ Open* **2026**, *16*, e102647. [CrossRef] [PubMed]
6. Britten-Jones, A.C.; Jin, R.; Gocuk, S.A.; Cichello, E.; O'Hare, F.; Hickey, D.G.; Edwards, T.L.; Ayton, L.N. The safety and efficacy of gene therapy treatment for monogenic retinal and optic nerve diseases: A systematic review. *Genet. Med.* **2022**, *24*, 521–534. [CrossRef] [PubMed]
7. Savastano, M.C.; Rizzo, C.; Fossataro, C.; Bacherini, D.; Giansanti, F.; Savastano, A.; Arcuri, G.; Rizzo, S.; Faraldi, F. Artificial intelligence in ophthalmology: Progress, challenges, and ethical implications. *Prog. Retin. Eye Res.* **2025**, *107*, 101374. [CrossRef] [PubMed]
8. Cione, F.; De Bernardo, M.; Di Stasi, M.; De Luca, M.; Albano, R.; Rosa, N. Lens Factor Choice in IOL Power Calculation after Laser Refractive Surgery: The Right Constant for Advanced Lens Measurement Approach (ALMA). *J. Clin. Med.* **2024**, *13*, 5186. [CrossRef] [PubMed]
9. Crincoli, E.; Savastano, M.C.; Savastano, A.; Carlà, M.M.; Giannuzzi, F.; Catania, F.; Kilian, R.; Rizzo, C.; Rizzo, S. Artificial intelligence and neurodegeneration: State of the art and explainability. *Int. J. Retin. Vitr.* **2026**. [CrossRef] [PubMed]
10. De Bernardo, M.; Vitiello, L.; Rosa, N. A-scan ultrasonography and optic nerve sheath diameter assessment during acute elevations in intra-abdominal pressure. *Surgery* **2020**, *167*, 1023–1024. [CrossRef] [PubMed]
11. De Bernardo, M.; Vitiello, L.; Capone, M.; Rosa, N. A-scan ultrasonography and optic nerve sheath diameter evaluation in children with acute liver failure. *Liver Int.* **2020**, *40*, 1504. [CrossRef] [PubMed]
12. De Bernardo, M.; Vitiello, L.; Rosa, N. Optic nerve sheath diameter ultrasonography in differentiation of ischemic and hemorrhagic strokes. *Am. J. Emerg. Med.* **2019**, *37*, 1384–1385. [CrossRef] [PubMed]

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Review

Precision Medicine in Inherited Retinal Disease: Advances, Challenges, and Future Directions

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Abstract

Background/Objectives: Inherited retinal diseases (IRDs) represent a group of rare conditions characterized by significant clinical and genetic heterogeneity. Historically, the diagnosis of these conditions relied primarily on clinical presentation and imaging techniques. This literature review aims to discuss the current state of progress and ongoing challenges in applying precision medicine approaches to IRDs and to examine how advances in genetic testing have transformed diagnostics and opened new therapeutic avenues. **Methods**: This review examines the application of genetic testing methods to IRDs, with particular focus on next-generation sequencing (NGS) technologies. The review also evaluates current patient selection protocols that combine genetic confirmation, retinal structural evaluation, and detailed genetic counselling to achieve optimal therapeutic outcomes. **Results**: The implementation of NGS has significantly enhanced diagnostic capabilities for IRDs by enabling precise identification of specific genetic mutations. This advancement has paved the way for targeted therapeutic strategies, exemplified by Luxturna for RPE65-related IRDs. However, several barriers to broader adoption of precision medicine persist, including high costs, varied access to services, and complexities in interpreting genetic variants. **Conclusions**: While the continued development of innovative therapeutic modalities offers promise for expanding treatment options for IRDs, fully harnessing the potential of current and emerging therapeutic technologies requires addressing existing economic, technological, educational, and infrastructural challenges.

Keywords: precision medicine; inherited retinal diseases gene therapies; retina

1. Introduction

Inherited retinal diseases (IRDs) are a group of rare, progressive conditions that can result in visual deficits or blindness due to dysfunction, degeneration, or abnormal development of the photoreceptors or the retinal pigment epithelium. IRDs encompass a wide range of disorders that differ in severity, age of onset, and rate of progression. They may affect central or peripheral vision and can present from early childhood to late adulthood. Although individually rare, IRDs are collectively important because of their chronic nature and long-term impact on vision and quality of life [1].

IRDs show significant clinical and genetic heterogeneity. For example, two patients with the same clinical diagnosis may experience different ages of onset, rates of progression, and severity of symptoms, while others with similar clinical presentations may have entirely different genetic mutations underlying their disease. These differences can come from principles such as allelic heterogeneity, where different mutations in the same gene can cause similar conditions, or locus heterogeneity, where mutations in different genes result in the same disease. This variability demonstrates why IRDs require a precision medicine approach [1].

In recent years, genetic testing has improved, changing the way clinicians approach IRDs. Rather than only relying on clinical features and imaging, there is now an increasing emphasis on identifying the genetic basis of disease in order to inform the diagnostic plan for the patient. This shift is one of the goals of precision medicine. Because IRDs are caused by a wide range of mutations across many genes, gene therapy aims to overcome this heterogeneity by targeting specific, disease-causing variants, either by replacing the defective gene, correcting the mutation, or modulating gene expression. This precision-based strategy enables more targeted and potentially effective treatments for patients [2].

The application of precision medicine for the treatment of IRDs is especially promising. However, understanding the results of genetic testing when variants of uncertain significance are involved is more challenging for doctors and patients. Exploring the techniques and methods used for interpreting IRDs and understanding the difficulty of linking genetic information with clinical outcomes is essential for advancements in precision medicine [2].

2. Background

The following section briefly outlines examples of IRDs to show this variability in clinical presentation and genetic mechanisms.

2.1. Retinitis Pigmentosa (RP)

Early signs of RP often include trouble seeing in low light (nyctalopia) and peripheral vision loss, which can later affect central vision as well. However, the age of onset and severity vary widely, and patients may also experience photophobia, near-sightedness, or colour vision defects. With over 80 genes implicated, RP can be inherited in autosomal dominant (20–25%), autosomal recessive (15–20%), or X-linked (10–15%) patterns, and rare cases of digenic inheritance have been reported. Even within the same gene, different mutations may have differing clinical presentations. For example, *ABCA4* and *BEST1* mutations can cause RP alongside other retinal dystrophies, and the same pathogenic variant can also present in a variety of ways across families [3].

2.2. Leber Congenital Amaurosis (LCA)

This IRD is a severe early-onset retinal dystrophy, which typically presents with visual impairment or blindness in infancy, though the severity of the disease and its symptoms, such as nystagmus, photophobia, or refractive errors, differ between patients. Additionally, fundus appearance and progression can differ, and extra-ocular features may occasionally be present in some patients. The condition is genetically diverse, with at least 20 genes involved, including *CEP290*, *CRB1*, *GUCY2D*, and *RPE65*. These genes affect different retinal pathways, such as phototransduction or ciliary function. Most cases are inherited in an autosomal recessive manner, though some rare forms follow an autosomal dominant pattern. This variation makes diagnosis complex, but also allows for new approaches to gene-specific therapy [4].

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2.3. Stargardt Disease (STGD)

STGD is the most common inherited macular dystrophy, varying in the age of onset, severity, fundus appearance, and progression [5]. Some individuals present in childhood with rapid vision loss and impairments, while others are diagnosed in adulthood with slower decline [6]. Yellow-white pisciform flecks and macular atrophy may be absent early on, complicating diagnosis. Furthermore, full-field ERG and imaging findings differ across patients [6]. STGD is most commonly caused by mutations in *ABCA4*, but even among patients with similar mutations, phenotypes can vary [3].

Due to the high level of variability seen among different genotypes implicated in IRDs and how the condition clinically presents, precision medicine is a promising method of therapy for these unique conditions [3].

3. Genetics (WES/WGS, VUS)

Diagnosing inherited retinal diseases has become more reliant on genetic testing, as these diseases usually have mutations across a wide range of genes. With the growing availability of gene panels, whole-exome sequencing (WES), and whole-genome sequencing (WGS), clinicians now have more methods to uncover the molecular basis of retinal degeneration. Moreover, the cost of sequencing, particularly for WGS, has decreased dramatically in recent years, falling from over $4000 to approximately $200 per genome, thereby increasing its accessibility for clinical diagnostic use. However, these advances have challenges, especially when genetic findings do not clearly explain a patient's symptoms. Variants of uncertain significance (VUS) further add to this complexity, making it hard to distinguish between benign variation and disease-causing mutations. At the same time, improved genetic resolution plays an important role in identifying patients eligible for new gene therapies, many of which are designed to target specific mutations or pathways involved in IRDs [7].

Gene panel sequences curated a list of genes known to be associated with IRDs. They are cost-effective and good for detecting known pathogenic variants, especially in populations with well-characterized mutation spectra. However, Panels can miss novel or rare variants outside the selected genes, which limits their use in highly heterogeneous populations or for discovering new disease genes [8].

WES sequences all protein-coding regions, capturing both known and novel variants across hundreds of IRD-associated genes [9]. WES is particularly valuable in genetically diverse populations or when initial gene panel testing is inconclusive [9]. It enables the discovery of new disease-causing genes and expands the mutation spectrum, which is seen in many IRD cohorts. However, WES may not detect deep intronic or regulatory variants and structural rearrangements [9].

WGS covers the entire genome, including coding, non-coding, and regulatory regions, and can identify variants which goes undetected by other methods [9]. WGS is being used more frequently for unresolved cases after panel or WES testing, although cost and data analysis still pose challenges [9].

Identifying clear links between different genetic variants and clinical features, known as genotype-phenotype correlations, is an important step in understanding inherited retinal diseases [10]. These correlations can allow researchers to understand how a disease might progress and help shape genetic counselling for families [10]. For example, individuals who carry two copies of a disease-causing mutation (homozygous variants) often present with more extreme symptoms or earlier onset when compared to individuals with only one copy (heterozygous) [10]. This pattern has been seen across multiple IRDs, including retinitis pigmentosa (RP), Leber congenital amaurosis (LCA), and Stargardt disease [10]. However, the relationship between genotype and phenotype is not always straightforward [10].

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Certain genes, such as *PROM1*, *USH2A*, and *ABCA4*, have been associated with a wide range of clinical outcomes [9]. Sometimes, the same gene can lead to differing conditions, which are dependent on the specific mutation involved and whether it is inherited in a dominant or recessive manner [9]. Despite the growing availability of genetic testing, strong genotype-phenotype matches are still only found in a fraction of patients [9]. One large study conducted in an Indian population, for example, reported clear correlations in just 35% of cases, which shows the ongoing challenges in interpreting gene data and the influence of other factors, such as modifier genes or environmental exposures, on clinical presentation [9]. A major hurdle in the genetic diagnosis of IRDs is the high number of variants that are classified as variants of uncertain significance (VUS) [11]. These are genetic changes for which there is not enough evidence to determine whether they are actually involved in causing disease or are simply benign differences [11]. In one study, nearly 45% of all IRD-related variants were categorized this way, emphasizing how often clinicians are left with ambiguous results [11]. This uncertainty complicates not only how clinicians approach diagnosis but also how they support patients in making sense of their results, as it is challenging to offer confident answers about prognosis, recurrence risk, or eligibility for gene-specific therapies [11]. Sometimes, added steps like functional assays, segregation studies in relatives, or analysis of variant frequency in populations that are diverse can help clarify whether a VUS is likely to be harmful [11]. Still, these follow-up investigations are not always possible, especially in resource-limited environments or when family members are unavailable [11].

Beyond VUS, several other factors make genetic diagnosis of IRDs complex. Many IRDs share overlapping clinical features, making it hard to tell them apart just from symptoms alone. At the same time, extensive genetic heterogeneity means that mutations in dozens to hundreds of different genes can lead to similar retinal findings. The situation becomes harder to untangle when considering population genetics. For example, genetic differences might be more frequently observed in specific communities or in areas with high rates of consanguinity, which affects both how common the disease is and how it appears in individuals. Despite growing reliance on newer sequencing technologies, a portion of IRD cases remain without a clear genetic explanation. This points to missing genes still to be discovered, deep intronic or structural variants not captured by standard tests, or more complex modes of inheritance that are not fully understood as of right now, and need further research [8].

4. Therapies and Trials

Luxturna (voretigene neparvovec), developed by Spark Therapeutics, is the first gene therapy approved by the FDA for any inherited disease, specifically targeting vision loss caused by biallelic mutations in the *RPE65* gene [11]. This therapy is indicated for patients with Leber congenital amaurosis (LCA) and certain forms of retinitis pigmentosa (RP) due to *RPE65* mutations [11].

With regard to its mechanism of action, Luxturna uses an adeno-associated viral (AAV2) vector to insert the *RPE65* gene into retinal cells via subretinal injection, restoring the ability to produce the critical enzyme required for the visual cycle [11]. In clinical trials, Luxturna led to significant and durable improvements in functional vision, light sensitivity, and visual function, with benefits persisting for at least three years [11]. Many patients were able to get back some independence, such as navigating without canes and recognizing faces [11]. The FDA approved Luxturna in December 2017 following unanimous advisory committee support, representing a major turning point in the treatment of inherited retinal diseases [12]. The approval of Luxturna has accelerated progress for therapies for other

IRDs. Several promising clinical trials are underway for genes implicated in RP, LCA, and related disorders:

RPGR (Retinitis Pigmentosa GTPase Regulator) mutations are a leading cause of X-linked retinitis pigmentosa. Multiple clinical trials are evaluating AAV-mediated gene replacement therapies for *RPGR*-associated RP [13]. Early results have shown some gains in visual function and safety, although long-term efficacy is still being assessed [14].

CEP290 mutations are the most common cause of LCA. Efforts to treat *CEP290* include both gene augmentation and antisense oligonucleotide approaches [14]. Notably, antisense therapy (such as sepofarsen) aims to correct splicing defects, and clinical trials have shown some improvement in vision in treated patients [14].

CRB1 mutations cause severe early-onset retinal dystrophies, including LCA and RP [14]. Preclinical studies and early-phase research trials are currently testing AAV-based gene replacement strategies for *CRB1*-related diseases [14].

Additional gene therapy trials are also happening for genes such as *CHM* (choroideremia), *USH2A* (Usher syndrome), *ABCA4* (Stargardt disease), and *RS1* (X-linked retinoschisis) [15]. Many of these therapies use AAV vectors, but alternative delivery systems and gene-editing approaches (such as CRISPR/Cas9) are also being looked into for genes with large coding sequences or dominant negative effects [15].

While challenges still remain for these therapies, as previously mentioned, precision medicine is a promising field of healthcare that could help better address the complications and complexities seen in IRDs and how individualized the condition is due to both its clinical and genetic heterogeneity seen among patients [15]. A summary of existing gene therapy preclinical and clinical trials is in Table 1.

Table 1. Clinical and Preclinical Trials for Gene Therapies in Inherited Retinal Diseases.

Trial/Program	Condition	Gene Target	Stage/Status	Organization/ Scientists	Trial/Literature Reference
AAV-RPE65 preclinical proof-of-concept (Briard dog)	RPE65-associated Leber congenital amaurosis/ inherited retinal degeneration	*RPE65*	Preclinical large-animal proof-of-concept; supported human translation	University of Pennsylvania/Cornell investigators	Acland et al., Nat Genet 2001 [16]
RPE65 Phase I gene transfer	RPE65-associated LCA/inherited retinal degeneration	*RPE65*	Completed Phase 1	NEI/University of Pennsylvania/CHOP and collaborators	NCT00481546; NCT00516477
Luxturna (voretigene neparvovec-rzyl; AAV2-hRPE65v2)	Biallelic RPE65 mutation-associated inherited retinal disease	*RPE65*	FDA approved Dec. 2017; pivotal Phase 3 completed	Spark Therapeutics	NCT00999609; Russell et al., Lancet 2017 [17]
Voretigene neparvovec long-term follow-up	Prior recipients of AAV2-hRPE65v2	*RPE65*	Long-term observational follow-up	Spark Therapeutics/Novartis	NCT03602820; NCT03597399
RPGRORF15 canine proof-of-concept	X-linked retinitis pigmentosa model	*RPGR*	Preclinical large-animal rescue study	University of Pennsylvania/collaborators	Beltran et al., PNAS 2012 [18]
Codon-optimized AAV8-RPGR	X-linked retinitis pigmentosa models	*RPGR*	Preclinical mouse-model optimization	University of Oxford/collaborators	Fischer et al., Mol Ther 2017 [19]

Table 1. *Cont.*

Trial/Program	Condition	Gene Target	Stage/Status	Organization/ Scientists	Trial/Literature Reference
AAV5-RPGR/botaretigene sparoparvovec Phase 1/2	RPGR-associated X-linked retinitis pigmentosa	*RPGR*	Completed Phase 1/2; safety and efficacy signals reported	MeiraGTx/Janssen	NCT03252847; Michaelides et al., Am J Ophthalmol 2024 [20]
BIIB112/cotoretigene toliparvovec Phase 1/2	RPGR-associated X-linked retinitis pigmentosa	*RPGR*	Completed Phase 1/2	Nightstar/Biogen	NCT03116113
LUMEOS Phase 3	RPGR-associated X-linked retinitis pigmentosa	*RPGR*	Phase 3; did not meet primary endpoint reported in 2025	Janssen/MeiraGTx	NCT04671433
AGTC-501/laruparetigene zovaparvovec Phase 1/2 (SKYLINE)	RPGR-associated X-linked retinitis pigmentosa	*RPGR*	Phase 1/2 clinical data published	AGTC/Beacon Therapeutics	NCT03316560; Yang et al., Am J Ophthalmol 2025 [21]
VISTA pivotal trial of AGTC-501/laruzova	RPGR-associated X-linked retinitis pigmentosa	*RPGR*	Randomized pivotal/registrational trial	Beacon Therapeutics	NCT04850118
LANDSCAPE bilateral-administration study	RPGR-associated X-linked retinitis pigmentosa	*RPGR*	Phase 2 open-label bilateral-treatment study	Beacon Therapeutics	NCT07174726
Sepofarsen/QR-110/AON-3 Phase 1b/2	CEP290-associated LCA10 due to c.2991+1655A>G (p.Cys998X)	CEP290	Completed Phase 1b/2 antisense oligonucleotide study	ProQR/Laboratoires Thea/Sepul Bio	NCT03140969; Russell et al., Nat Med 2022 [17]
ILLUMINATE sepofarsen Phase 2/3	CEP290-associated LCA10	*CEP290*	Completed Phase 2/3	ProQR	NCT03913143
HYPERION sepofarsen Phase 3	CEP290-associated LCA10	*CEP290*	Phase 3; first participant dosed reported in 2025	Sepul Bio/ Laboratoires Thea	NCT06891443
EDIT-101 BRILLIANCE	LCA10 due to CEP290 c.2991+1655A>G/ IVS26 mutation	*CEP290*	Completed Phase 1/2 in vivo CRISPR-Cas9 editing trial	Editas Medicine	NCT03872479; Pierce et al., N Engl J Med 2024 [22]
AAV2-REP1 first-in-human choroideremia gene therapy	Choroideremia	*CHM/REP1*	Completed Phase 1/2 dose-escalation	University of Oxford/Nightstar	NCT01461213; MacLaren et al., Lancet 2014 [23]
STAR: BIIB111/AAV2-REP1/timrepigene emparvovec	Choroideremia	*CHM/REP1*	Completed Phase 3; primary endpoint not met	Nightstar/Biogen	NCT03496012; Bergland et al., Nat Med 2023 [24]
BIIB111 bilateral-administration safety study	Choroideremia	*CHM/REP1*	Completed safety study	Nightstar/Biogen	NCT03507686
BIIB111 long-term follow-up	Prior BIIB111-treated choroideremia participants	*CHM/REP1*	Long-term safety and efficacy follow-up	Biogen	NCT03584165

Table 1. *Cont.*

Trial/Program	Condition	Gene Target	Stage/Status	Organization/ Scientists	Trial/Literature Reference
4D-110 intravitreal CHM gene therapy	Choroideremia	*CHM/REP1*	Phase 1 dose-escalation	Four-dimensional Molecular Therapeutics/Roche	NCT04483440
STELLAR: QR-421a/ultevursen	Usher syndrome type 2A/nonsyndromic RP due to USH2A exon 13 mutations	*USH2A*	Completed Phase 1/2 RNA exon-skipping trial	ProQR	NCT03780257
SIRIUS: ultevursen	Advanced RP due to USH2A exon 13 mutations	*USH2A*	Phase 2/3; terminated/transitioned after program changes	ProQR	NCT05158296
CELESTE: ultevursen	Early-to-moderate RP due to USH2A exon 13 mutations	*USH2A*	Phase 2/3; terminated/transitioned after program changes	ProQR	NCT05176717
LUNA: ultevursen	RP due to USH2A exon 13 mutations	*USH2A*	Recruiting Phase 2b, randomized sham-controlled	Sepul Bio/ Laboratoires Thea	NCT06627179
SAR422459/EIAV-ABCA4	ABCA4-associated Stargardt disease	*ABCA4*	Completed Phase 1/2a lentiviral gene therapy	Sanofi/Oxford BioMedica/OHSU/ Quinze-Vingts	NCT01367444; Parker et al., Am J Ophthalmol 2022 [25]
ASTRA: SB-007 dual-AAV protein-splicing therapy	ABCA4-associated Stargardt disease	*ABCA4*	Ongoing Phase 1/2; dose-expansion initiated	SpliceBio	NCT06942572
CELESTE: AAVB-039 dual-AAV ABCA4 therapy	ABCA4-associated Stargardt disease	*ABCA4*	Phase 1/2, multicenter open-label dose-escalation	AAVantgarde Bio	NCT07161544
VG801 mRNA trans-splicing gene therapy	ABCA4 mutation-associated recessive retinal dystrophy/ Stargardt disease	*ABCA4*	Phase 1/2 first-in-human dose-exploration	VeonGen/ViGeneron	NCT07002398
OCU410ST/AAV5-hRORA modifier gene therapy	Stargardt disease/ABCA4-associated retinopathy	*ABCA4* pathway modifier, not ABCA4 replacement	Phase 2/3 pivotal modifier-gene trial	Ocugen	NCT05956626
AAV8-RS1 ocular gene transfer	X-linked retinoschisis	*RS1*	Completed Phase 1/2a intravitreal trial; safety/ tolerability focus	National Eye Institute	NCT02317887; Cukras et al., Mol Ther 2018 [26]
rAAV2tYF-CB-hRS1	X-linked retinoschisis	*RS1*	Completed Phase 1/2 intravitreal trial; no measurable treatment effect	AGTC/OHSU and collaborators	NCT02416622; Pennesi et al., Ophthalmol Retina 2022 [27]
AAV2/4-RS1	X-linked retinoschisis model	*RS1*	Preclinical Rs1-KO mouse rescue study	University of Florida/collaborators	Scruggs et al., Mol Ther Methods Clin Dev 2022 [28]

Table 1. *Cont.*

Trial/Program	Condition	Gene Target	Stage/Status	Organization/ Scientists	Trial/Literature Reference
LIGHTHOUSE: ATSN-201	RS1-associated X-linked retinoschisis	*RS1*	Phase 1/2/3; pivotal Phase 3 cohort underway in 2026	Atsena Therapeutics	NCT05878860
PIONEER: GS030-DP/ChrimsonR optogenetic therapy	Advanced nonsyndromic retinitis pigmentosa	Mutation-independent optogenetics; retinal ganglion-cell ChrimsonR	Phase 1/2a; first reported partial functional recovery	GenSight Biologics/ Institut de la Vision	NCT03326336; Sahel et al., Nat Med 2021 [29]
ChR2 optogenetic preclinical studies	Rodent/canine retinal degeneration models	Mutation-independent optogenetics; *ChR2*	Preclinical proof-of-concept	Multiple academic groups	Gaub et al., PNAS 2014 [30]
ChrimsonR nonhuman-primate proof-of-concept	Late-stage retinal degeneration platform development	Mutation-independent optogenetics; ChrimsonR/ ChrimsonR-tdT	Preclinical nonhuman-primate safety and functional-expression study	GenSight/Sorbonne/ Institut de la Vision collaborators	Gauvain et al., Commun Biol 2021 [31]
RESTORE/MCO-010 optogenetic therapy	Advanced retinitis pigmentosa	Mutation-independent optogenetics; bipolar-cell MCO	Phase 2b randomized sham-controlled study; positive durability reports	Nanoscope Therapeutics	NCT04945772; Lam et al., Nat Med/PMC 2025 [32]

5. Patient Selection

Clinically using gene therapy in IRDs requires a balance of eligibility and ethical considerations [33]. Precision medicine begins with patient selection protocols that are both biologically and ethically justified, all while managing patients and their families regarding the complexity, limitations and implications of therapy [33].

Eligibility for gene therapy requires, first, a genetic confirmation of disease etiology, primarily in the form of biallelic pathogenic variants in a causative gene [33]. As previously discussed, treatment with Luxturna requires two disease-causing mutations in the RPE65 gene, as established by FDA and EMA criteria [34]. Diagnoses often utilize NGS gene panels due to their efficiency and broad coverage across the genetic spectrum of IRDs [33]. The American Academy of Ophthalmology (AAO) recommends the inclusion of syndromic genes in all IRD panels, given the possibility of delayed systemic involvement, which further leverages the strength of NGS [33]. To reiterate, panels of patients with nonsyndromic presentations also include testing for syndromic genes, as systemic features such as hearing loss in Usher syndrome may emerge only later in life [33]. Furthermore, the accurate interpretation of results, especially in VUS, depends on the analytic sensitivity, quality of the reference database, and expert interpretation, underscoring the importance of collaboration among ophthalmologists, molecular laboratories, and certified genetic counsellors [35].

In addition to the presence of relevant genetic mutations, candidates may also need to demonstrate some level of preserved retinal architecture, as gene therapy cannot regenerate lost photoreceptors [36]. At this stage of testing, optical coherence tomography (OCT) is used to evaluate retinal structure, specifically assessing the continuity of the ellipsoid zone and central retinal thickness [36]. These measures are surrogates for photoreceptor

viability and have been correlated with improved visual outcomes following subretinal gene delivery [37]. Full-field electroretinography (ERG) is also used to assess the function of rods and cones, supporting diagnosis and disease staging [37]. The quantitative readout provided by full-field ERG is crucial in confirming retinal involvement, especially in conditions such as LCA and RP [33]. This is an especially sensitive and vital test: even in patients with localized macular involvement, full-field ERG can detect subclinical, widespread degeneration that may affect outcomes and eligibility [33]. Furthermore, kinetic and static visual field testing can be used to help determine disease severity and baseline function, identifying patients who fall within regulatory or trial criteria, such as thresholds for legal blindness [33]. This can also serve as a measure of functional outcomes in trials [33]. Finally, fundus autofluorescence (FAF) and infrared reflectance imaging may support the identification of metabolically active RPE; however, FAF should be used with caution in macular dystrophies such as Stargardt disease, where short-wavelength exposure poses a theoretical risk of light toxicity [38].

Due to the progressive and irreversible loss of photoreceptors in IRD, treatment timing is critical, and regulatory approvals provide age-specific frameworks [38]. As explored earlier, Luxturna is currently intended for patients 12 months of age and older [39]. This is based on Phase 3 trial data and long-term follow-up studies, which indicate better visual outcomes in younger patients with less advanced disease [39]. Most ongoing trials in IRDs set age limits to account for the reliability of the test, disease progression and surgical risk [39]. For pediatric candidates, additional considerations include the feasibility of sedated imaging and ERG, as well as the challenges in assessing visual acuity and fields [39]. Clinical trials of different gene therapies may apply more stringent inclusion criteria [39]. The ongoing EDIT-101 trials for CEP290-related Leber congenital amaurosis (LCA10) include only individuals with a certain margin of residual retinal structure, as assessed by OCT [40]. Similarly, clinical trials targeting *RPGR*, *CRB1*, and *CHM* genes may also incorporate additional requirements, such as visual acuity thresholds, preservation of macular function, or exclusion of individuals who have undergone prior ocular surgeries [40].

Beyond initial selection, patients who are candidates for gene therapy require structured long-term follow-ups to assess safety, functional outcomes and disease progression [33]. Retinal imaging, ERF and visual field testing are periodically repeated to monitor the effect of treatment and detect late-onset complications [33]. These can include inflammation and subretinal fibrosis [33]. Clinicians, then, should prepare patients for immediate procedure and a lifelong trajectory of follow-up, re-evaluation and emotional adaptation [33].

This leads to a final, and perhaps most important, aspect of patient selection: comprehensive genetic counselling [33]. The importance of genetic counselling cannot be overstated as an essential and ethical part of patient selection [33]. In both trials and clinical use, patients express both hope and anxiety regarding gene therapy decisions [33]. As such, patients and families may assume curative outcomes. Instead, counselling helps reframe expectations towards stabilization or modest improvement [33]. For instance, in the Luxturna trials, although improved functional vision was observed in many patients, outcomes varied depending on age, disease stage, and baseline function [41]. Genetic counselling is especially vital in IRD, where VUS are frequently encountered and more than 80 genes can contribute to overlapping phenotypes [41]. Here, results require careful and nuanced interpretation and explanation to avoid inappropriate exclusion of false hope [41]. The AAO recommends that post-test counselling be conducted by clinicians or geneticists familiar with ocular genomics and ideally be reoffered throughout life as new therapies and variant classifications evolve [33]. Counselling must address psychosocial dynamics that

patients and patient families often grapple with, such as feelings of hope, fear, guilt, and grief [33]. These may emerge both pre- and post-therapy, especially in families navigating decisions for children or those living with a progressive disability [33]. Support must not end with the disclosure of test results, and longitudinal counselling (and re-counselling) align with the best ethical practices to support informed and supported autonomy [33].

6. Barriers

Although gene therapy holds promising advancements for IRDs, several barriers must be overcome before it can be widely implemented [33]. These barriers span economic, infrastructure, technical and informational aspects [33].

6.1. Economic

Firstly, the high cost of gene therapy presents a significant barrier to access [42]. In the United States, Luxturna is priced at approximately $850,000 for both eyes, posing challenges at both the system and patient levels [42]. From the insurers' perspective, health technology assessments are complicated by the high upfront cost coupled with uncertain outcomes [42]. These problematize payers' coverage and affect access to gene therapy [43]. However, even when insurance is available, IRD patients often face restrictive costs, especially within the broader scope of the economic burden associated with IRDs [43]. Children with IRDs have 40% higher health expenses than their peers, owing mainly to increased outpatient eye care and psychiatric services [44]. The lifetime cost per person with IRD was estimated at $5.2 million in Australia, with 87% of the cost arising from societal costs, including loss of income for both patients and their caregivers [45]. Unfortunately, such financial burdens and patterns often persist into adulthood for those with IRDs as well [45]. While employment rates for adults with IRDs in Singapore are similar to those of the general population, the income for those with IRDs remains significantly lower [46]. In Canada, individuals with IRDs are 24% less likely to hold paid employment compared to the national average [47]. Furthermore, the estimated per-person societal costs associated with IRDs in Canada range from US$16,470 to over US$275,000 annually [47]. Lost productivity, diminished quality of life, and informal caregiving responsibilities contribute to up to 98% of these costs [48]. Thus, financial inaccessibility encompasses not only the high price of treatment but also the broader economic vulnerability experienced by individuals living with IRDs [48].

6.2. Infrastructural

Furthermore, the actual availability and distribution of centres and trained professionals vary globally and influence accessibility [49]. In Portugal, for example, genetic testing was found to be only available in 54.5% of centres, and of the 26 centres studied, only four actually used the national IRD registry [49]. Moreover, 83.4% of IRD patients experienced a turnaround time of 4 to 9 months for genetic test results [49]. Similarly, in Italy, patients with RPE65-related IRDs often consulted up to 10 healthcare professionals before finding an appropriate specialized centre [49]. This highlights that even when a centre is available, in the absence of a well-organized referral network, barriers to diagnosis and treatment persist [50]. Such a trend is also prevalent in Canada, where a study of 408 patients and caregivers found challenges in accessing genetic testing and specialized care, with many patients reporting delays in diagnosis and treatment [51]. While there are more specialized centres for IRD gene therapy in the United States, access remains uneven [51]. Centres such as the Bascom Palmer Eye Institute and the Massachusetts Eye and Ear Infirmary are concentrated in specific regions, which may lead to disparities in access for patients residing in rural or underserved areas [51].

6.3. Technical

Even once a centre is located, certain barriers persist [52]. The success of gene therapy in IRDs is dependent on the precise delivery and sustained expression of the therapeutic gene. As touched on briefly, variability in vector transduction efficiency, potential immune response and need for viable target cells can complicate treatment outcomes [52]. For example, the efficiency of AAV vectors can be affected by the presence of pre-existing neutralizing antibodies that can vary among individuals [52]. In some cases, the introduction of viral vectors can activate the immune response and cause inflammation, leading to a potential reduction in efficacy [52]. In the context of IRDs, these immune reactions can exacerbate retinal degeneration and present as gene therapy-associated uveitis (GTU), which has been reported in clinical trials [53]. In regard to the aspect of viable target cells, identifying patients early is crucial for the effective implementation of therapy. Leber congenital amaurosis and achromatopsia, for example, present preserved retinal structure for extended periods and are more ideal candidates for gene therapy when compared to an IRD such as retinitis pigmentosa, which presents with rapid retinal degeneration [54–56].

Informational

On a broader level, a lack of awareness and a clear understanding of gene therapy among patients and healthcare providers can further impede its implementation [57]. Misconceptions and limited access to accurate information may lead to barriers at a societal and mental level [57]. An Australian survey by Mack et al. probing the perspectives of individuals with IRDs found that only 28.3% of participants agreed they have good knowledge of gene therapy [57]. Though 86.9% were able to differentiate between an experimental treatment and an approved therapy, 52.9% incorrectly believed that gene therapy and stem cell therapy are the same. In a study with similar themes by Britten-Jones et al., only 37% of participants correctly responded that gene therapy for the eye is not suitable for all stages of the disease, and only 48% knew that the primary goal of the therapy is to slow the progression of the disease [58]. Such knowledge gaps are also observed in healthcare providers themselves [58]. A survey of optometrists in Australia and New Zealand found that only 38% of participants correctly identified the primary goal of gene therapy (slowing the progression of disease), and 81% indicated a lack of clarity on referral pathways as a key barrier [59].

Knowing this, efforts to address informational gaps are crucial for supporting decision-making and improving the implementation of gene therapy [58]. Britten-Jones et al. also found that individuals who self-reported good knowledge of genetic therapy were approximately three times more likely to feel confident in managing their own or their dependent's eye care following testing [58]. One resource to enhance patient education is the Foundation Fighting Blindness annual VISION conference for patients and families [47]. The conference offers a place to connect with the community, learn about the latest research and treatments and explore new clinical trials and products within a supportive environment [47].

7. Future Directions

Gene therapy, as an emerging technology, still faces some degree of limitations—whether that be immunogenicity, restricted vector capacity or concerns about long-term efficacy—and so fosters a centre for further innovation. CRISPR-Cas9 gene editing, antisense oligonucleotides (ASOs), optogenetics and multi-omics approaches all hold potential, with each technology harbouring its own advantages and challenges, a few of which are discussed below [60].

7.1. CRISPR-Cas9

CRISPR-Cas9 is a two-component genome-editing system founded on a single-guide RNA (sgRNA) directing a Cas9 endonuclease to specific DNA sequences in order to generate a site-specific double-strand break (DSB) [60]. This break is then repaired by the cell and results in targeted insertions, deletions or precise replacements [60].

Applying CRISPR-Cas9 to IRDs has accelerated, originally beginning with using animal models with relevant mutations to aid in developing therapeutics that can edit photoreceptor or RPE cells in vivo [61]. A landmark preclinical study by Maeder et al. explored EDIT-101, an AAV-delivered CRISPR-Cas9 system aiming to edit the mutation responsible for LCA10 [61]. EDIT-101 achieved sustained editing rates, even exceeding the presumed therapeutic threshold and restoring regular transcript expression for photoreceptor structure and function [61].

Since then, a Phase ½ clinical trial (BRILLIANCE) has been evaluating the safety and tolerability of subretinally delivered EDIT-101 in LCA10 patients [22]. Early interim data thus far have indicated on-target editing in patient-derived retinal cells and no serious adverse events. Though final data is pending, these findings support the feasibility of CRISPR-Cas9 therapeutics in the field of IRDs [22].

At its ideal, CRISPR-Cas9 for IRDs can include the potential for permanent correction of recessive or dominant-negative mutations with a single intervention, standing to eliminate the need for repeat dosing [62]. Furthermore, the programmable nature of sgRNAs offers precise targeting of mutations across diverse IRD-implicated genes [62]. However, off-target cleavage remains a concern: unintended DSBS can cause changes in nontarget loci with variable downstream effects [62]. Preexisting or induced immune responses against the bacterial Cas9 protein could also reduce efficacy and lead to retinal inflammation [62]. Finally, efficient delivery to photoreceptors or RPE cells requires optimized vectors, and the current usage of AAV introduces constraints for the inclusion of larger Cas9 orthologs [63].

That being said, CRISPR-Cas9 editing in other genetic diseases, such as sickle cell disease, has demonstrated reliable on-target gene correction and clinical benefit [64]. For applicable translation to IRDs, general improvements in the CRISPR field can also be applied, such as engineering high-fidelity Cas9 variants, smaller Cas9 orthologs with AAV and the development of non-viral delivery methods [65].

7.2. ASOs

Antisense oligonucleotides, or ASOs, are short synthetic strands of nucleic acids that can hybridize with specific pre-mRNA sequences to modulate splicing [66]. In IRDs, ASOs aim to bind to mutated exons and induce exon skipping, allowing the synthesis of a truncated but partially functional protein [66]. Dulla et al. demonstrated that an intravitreal delivery of ASOs in a murine retinitis pigmentosa model successfully skipped the mutated exon [66]. This resulted in a 35–45% restoration of full-length transcription and partial preservation of photoreceptor function [66].

Several ASO candidates for *CEP290*-related LCA are in the early stages of clinical development [67]. AON-3, now formulated as QR-110 or sepofarsen, targets the c.2991+1655A>G *CEP29*-mutation and was found to restore normal *CEP290* mRNA in patient-derived retinal organoids [67]. Based on these findings, a first-in-human trial is evaluating safety and tolerability in *LCA10* patients [67]. Interim data describe dose-dependent editing in patient-derived retinal cells and no serious adverse events attributable to Cas9 editing [17].

ASOs offer high specificity, and they can be designed with single-nucleotide precision [17,67]. Unlike permanent gene editing, the effects of ASO are reversible; ASO gradually degrades over weeks, allowing for dosing adjustments if adverse effects arise [17,67]. On the flip side of the same coin, however, ASO therapy requires repeated intravitreal

injections to maintain therapeutic levels, leading to increased procedural burden and risks [17,67]. It is also worth noting that exon skipping often yields retention of only partial function, and off-target splicing effects are also possible [17,67]. However, in IRDs, even partial preservation could maintain functional vision for years, and thus, ASOs present a compelling intermediate between gene supplementation and permanent editing [17,67].

7.3. Optogenetics

Optogenetics aims to repurpose surviving inner retinal neurons (typically bipolar or ganglion cells) into light detectors by using microbial opsin genes [29]. Sahel et al. report a successful case [29]. A patient presenting with retinitis pigmentosa received an intravitreal injection with an *AAV2* vector encoding a red-shifted channelrhodopsin, ChrimsonR, under a bipolar-cell-specific promoter [29]. When the patient then wore specifically designed amber-filtered goggles, he was able to locate and count objects, detect moving people, and recognize high-contrast shapes, regaining abilities he had lost prior to treatment [29]. These abilities persisted at six months without significant inflammation or adverse events [29].

Preclinical work in nonhuman primates has confirmed that bipolar-cell-specific expression of ChrimsonR via *AAV2* can yield stable mutation-independent photoreception [68]. A canine model of progressive rod-cone degeneration found that intravitreal delivery of AAV2-ChR2 restored light-driven pupillary reflexes and coordination to moving stimuli [68].

It is worth noting that while intravitreal injection is less invasive than subretinal surgery, visual acuity remains low, and bulky goggles are required to deliver sufficiently intense, wavelength-specific light [29,68]. Device calibration and patient training can take several weeks to achieve reliable performance [29,68]. However, optogenetics offers one of the few viable near-term options for patients lacking residual photoreceptor functions and could be combined with future cell-replacement or gene-correction approaches [29,68].

7.4. Multi-Omics

Multi-omics integrates multiple molecular layers (genome, transcriptome, proteome, metabolome and epigenome) to build a comprehensive molecular picture of disease [69]. In IRDs, multi-omics has been employed to elucidate complex genotype-phenotype relationships and identify novel biomarkers for diagnosis and stratification [69]. Liang et al. generated a single-nucleus multi-omics atlas of adult human retinas, combining transcriptomics and chromatin accessibility profiles from more than 250,000 nuclei [69]. Integrating this data revealed a core module of IRD-associated genes, including photoreceptor-specific transcription factors and metabolic enzymes, which had not been apparent from genetics alone [69]. Similarly, Lei et al. applied proteomic and metabolomic profiling to the vitreous samples of RP patients to identify elevated oxidative stress markers and dysregulated lipid pathways, suggesting potential targets for neuroprotection [70].

Advantages of multi-omics include the sensitive detection of subclinical disease stages, the refinement of patient subgroups for clinical trials, and the discovery of drug-target pathways [69,70]. However, this approach is resource-intensive, comprising sample collection, high-throughput sequencing, mass spectrometry, and computational integration [69,70]. From a patient's perspective, multi-omics can yield personalized prognoses and inform the tailoring of future therapies [69,70]. However, broad implementation hinges on developing streamlined and cost-effective assays [69,70]. In practice, multi-omics is thus an emerging, realistic adjunct to genetic testing, with the power to accelerate the diagnosis of unsolved IRDs and prioritize patients for mutation-specific interventions [69,70].

Lastly, prior to implementing precision medicine in IRD therapeutic strategies, safety and procedural risks should be explored and addressed [71]. A recent systematic review

and meta-analysis found that AAV ocular gene therapy had a cumulative serious adverse event incidence of 8%, with most adverse events attributable to the surgical procedure used to deliver the vector [71]. Ocular inflammation related to high-dose ocular gene therapies was found to be generally transient and resolved upon administration of corticosteroids [71]. Despite these findings, adverse event reporting in ocular gene therapy trials is inconsistent, suggesting that adverse events may occur at a higher rate than reported [71]. Future trials should investigate methods for safe dose selection and optimal delivery, such as intraoperative assistance with optical coherence tomography, to avoid off-target exposure, inflammation, and mechanical injury to the retina [71].

8. Conclusions

Over the past decade, precision medicine has transformed the IRD landscape: NGS panels and complementary imaging modalities now enable genetic diagnoses in the majority of patients, and multi-omics approaches and functional assays deepen our understanding of variant pathogenicity. Informed patient-selection protocols have already yielded tangible clinical benefits, and treatments are increasingly emerging and becoming available.

Despite these advancements, certain challenges persist. A marked subset of IRD cases remains genetically unresolved due to the presence of deep-intronic, structural, or non-coding variants that elude standard assays. Long-term safety and efficacy data for gene-based intervention are still maturing, and high costs, bioinformatic needs and regional disparities continue to limit broad, equitable access.

To bridge these gaps, the field must develop efficient and effective technology pipelines, in addition to forging international consortia to harmonize patient registries, trial eligibility, and post-market surveillance. Sustaining collaboration among geneticists, clinicians, health economists, patient advocates, and policymakers, the promise of precision medicine can be leveraged to translate into durable, accessible treatment; ultimately, to give the gift of sight to as many IRD patients as possible.

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Abbreviations

The following abbreviations are used in this manuscript:

Abbreviation	Full Term
AAV	Adeno-associated virus
ABCA4	ATP Binding Cassette Subfamily A Member 4
AON	Antisense oligonucleotide
ASO	Antisense oligonucleotide
BEST1	Bestrophin 1
CEP290	Centrosomal Protein 290

CHM	Choroideremia
CRB1	Crumbs Cell Polarity Complex Component 1
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DNA	Deoxyribonucleic acid
ERG	Electroretinography
FAF	Fundus autofluorescence
FDA	Food and Drug Administration
GUCY2D	Guanylate Cyclase 2D
IRDs	Inherited retinal diseases
LCA	Leber congenital amaurosis
NGS	Next-generation sequencing
OCT	Optical coherence tomography
PROM1	Prominin 1
RNA	Ribonucleic acid
RP	Retinitis pigmentosa
RPE65	Retinal pigment epithelium-specific 65 kDa protein
RPGR	Retinitis Pigmentosa GTPase Regulator
RS1	Retinoschisin 1
STGD	Stargardt disease
USH2A	Usherin
VUS	Variants of uncertain significance
WES	Whole exome sequencing
WGS	Whole genome sequencing

References

1. Ben-Yosef, T. Inherited Retinal Diseases. *Int. J. Mol. Sci.* **2022**, *23*, 13467. [CrossRef]
2. Naithani, N.; Sinha, S.; Misra, P.; Vasudevan, B.; Sahu, R. Precision medicine: Concept and tools. *Med. J. Armed Forces India* **2021**, *77*, 249–257. [CrossRef]
3. Bravo-Gil, N.; González-Del Pozo, M.; Martín-Sánchez, M.; Méndez-Vidal, C.; Rodríguez-de la Rúa, E.; Borrego, S.; Antiñolo, G. Unravelling the genetic basis of simplex Retinitis Pigmentosa cases. *Sci. Rep.* **2017**, *7*, 41937. [CrossRef]
4. Huang, C.H.; Yang, C.M.; Yang, C.H.; Hou, Y.C.; Chen, T.C. Leber's Congenital Amaurosis: Current Concepts of Genotype-Phenotype Correlations. *Genes* **2021**, *12*, 1261. [CrossRef] [PubMed]
5. Tanna, P.; Strauss, R.W.; Fujinami, K.; Michaelides, M. Stargardt disease: Clinical features, molecular genetics, animal models and therapeutic options. *Br. J. Ophthalmol.* **2017**, *101*, 25–30. [CrossRef]
6. Cremers, F.P.M.; Lee, W.; Collin, R.W.J.; Allikmets, R. Clinical spectrum, genetic complexity and therapeutic approaches for retinal disease caused by ABCA4 mutations. *Prog. Retin. Eye Res.* **2020**, *79*, 100861. [CrossRef]
7. Christensen, K.D.; Dukhovny, D.; Siebert, U.; Green, R.C. Assessing the Costs and Cost-Effectiveness of Genomic Sequencing. *J. Pers. Med.* **2015**, *5*, 470–486. [CrossRef]
8. Chen, Z.J.; Lin, K.H.; Lee, S.H.; Shen, R.J.; Feng, Z.K.; Wang, X.F.; Huang, X.F.; Huang, Z.Q.; Jin, Z.B. Mutation spectrum and genotype-phenotype correlation of inherited retinal dystrophy in Taiwan. *Clin. Exp. Ophthalmol.* **2020**, *48*, 486–499. [CrossRef]
9. Fan, X.; Li, Z.; Sha, L.; Sheng, X.; Rong, W. Genotype-phenotype correlations for 17 Chinese families with inherited retinal dystrophies due to homozygous variants. *Sci. Rep.* **2025**, *15*, 3043. [CrossRef] [PubMed]
10. Gopinath, C.; Rompicherla, R.; Mathias, G.P.; Patil, R.; Poornachandra, B.; Vinekar, A.; Mochi, T.B.; Braganza, S.; Shetty, K.B.; Kumaramanickavel, G.; et al. Inherited retinal disorders: A genotype-phenotype correlation in an Indian cohort and the importance of genetic testing and genetic counselling. *Graefes Arch. Clin. Exp. Ophthalmol.* **2023**, *261*, 2003–2017. [CrossRef] [PubMed]
11. Trapani, I.; Auricchio, A. Seeing the Light after 25 Years of Retinal Gene Therapy. *Trends Mol. Med.* **2018**, *24*, 669–681. [CrossRef]
12. Shaberman, B. A Retinal Research Nonprofit Paves the Way for Commercializing Gene Therapies. *Hum. Gene Ther.* **2017**, *28*, 1118–1121. [CrossRef]
13. MedlinePlus. Leber Congenital Amaurosis. 2022. Available online: https://medlineplus.gov/genetics/condition/leber-congenital-amaurosis/ (accessed on 5 September 2025).
14. Alyea, G.; Francis, P.; Mansfield, B.; Focus, H.I. Leber Congenital Amaurosis. Available online: https://rarediseases.org/rare-diseases/leber-congenital-amaurosis/ (accessed on 5 September 2025).

15. Ameri, H.; Kesavamoorthy, N.; Bruce, D.N. Frequency and Pattern of Gene Therapy Clinical Trials for Inherited Retinal Diseases. *Adv. Exp. Med. Biol.* **2025**, *1468*, 89–93. [CrossRef]
16. Acland, G.M.; Aguirre, G.D.; Ray, J.; Zhang, Q.; Aleman, T.S.; Cideciyan, A.V.; Pearce-Kelling, S.E.; Anand, V.; Zeng, Y.; Maguire, A.M.; et al. Gene therapy restores vision in a canine model of childhood blindness. *Nat. Genet.* **2001**, *28*, 92–95. [CrossRef] [PubMed]
17. Russell, S.R.; Drack, A.V.; Cideciyan, A.V.; Jacobson, S.G.; Leroy, B.P.; Van Cauwenbergh, C.; Ho, A.C.; Dumitrescu, A.V.; Han, I.C.; Martin, M.; et al. Intravitreal antisense oligonucleotide sepofarsen in Leber congenital amaurosis type 10: A phase 1b/2 trial. *Nat. Med.* **2022**, *28*, 1014–1021. [CrossRef]
18. Beltran, W.A.; Cideciyan, A.V.; Lewin, A.S.; Iwabe, S.; Khanna, H.; Sumaroka, A.; Chiodo, V.A.; Fajardo, D.S.; Román, A.J.; Deng, W.-T.; et al. Gene therapy rescues photoreceptor blindness in dogs and paves the way for treating human X-linked retinitis pigmentosa. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 2132–2137. [CrossRef]
19. Fischer, M.D.; McClements, M.E.; Martinez-Fernandez de la Camara, C.; Bellingrath, J.S.; Dauletbekov, D.; Ramsden, S.C.; Hickey, D.G.; Barnard, A.R.; MacLaren, R.E. Codon-Optimized RPGR Improves Stability and Efficacy of AAV8 Gene Therapy in Two Mouse Models of X-Linked Retinitis Pigmentosa. *Mol. Ther.* **2017**, *25*, 1854–1865. [CrossRef]
20. Michaelides, M.; Besirli, C.G.; Yang, Y.; TAC, D.E.G.; Wong, S.C.; Huckfeldt, R.M.; Comander, J.I.; Sahel, J.A.; Shah, S.M.; Tee, J.J.L.; et al. Phase 1/2 AAV5-hRKp.RPGR (Botaretigene Sparoparvovec) Gene Therapy: Safety and Efficacy in RPGR-Associated X-Linked Retinitis Pigmentosa. *Am. J. Ophthalmol.* **2024**, *267*, 122–134. [CrossRef] [PubMed]
21. Yang, P.; Birch, D.; Lauer, A.; Sisk, R.; Anand, R.; Pennesi, M.E.; Iannaccone, A.; Yaghy, A.; Scaria, A.; Jung, J.A.; et al. Subretinal Gene Therapy Drug AGTC-501 for XLRP Phase 1/2 Multicenter Study (HORIZON): 24-Month Safety and Efficacy Results. *Am. J. Ophthalmol.* **2025**, *271*, 268–285. [CrossRef] [PubMed]
22. Pierce, E.A.; Aleman, T.S.; Jayasundera, K.T.; Ashimatey, B.S.; Kim, K.; Rashid, A.; Jaskolka, M.C.; Myers, R.L.; Lam, B.L.; Bailey, S.T.; et al. Gene Editing for CEP290-Associated Retinal Degeneration. *N. Engl. J. Med.* **2024**, *390*, 1972–1984. [CrossRef]
23. MacLaren, R.E.; Groppe, M.; Barnard, A.R.; Cottriall, C.L.; Tolmachova, T.; Seymour, L.; Clark, K.R.; During, M.J.; Cremers, F.P.; Black, G.C.; et al. Retinal gene therapy in patients with choroideremia: Initial findings from a phase 1/2 clinical trial. *Lancet* **2014**, *383*, 1129–1137. [CrossRef] [PubMed]
24. Bergland, H.; Burlakov, E.; Wyller, J. The Dynamics of Pasture-Herbivores-Carnivores with Sigmoidal Density Dependent Harvesting. *Bull. Math. Biol.* **2023**, *85*, 104. [CrossRef]
25. Parker, M.A.; Erker, L.R.; Audo, I.; Choi, D.; Mohand-Said, S.; Sestakauskas, K.; Benoit, P.; Appelqvist, T.; Krahmer, M.; Ségaut-Prévost, C.; et al. Three-Year Safety Results of SAR422459 (EIAV-ABCA4) Gene Therapy in Patients with ABCA4-Associated Stargardt Disease: An Open-Label Dose-Escalation Phase I/IIa Clinical Trial, Cohorts 1-5. *Am. J. Ophthalmol.* **2022**, *240*, 285–301. [CrossRef]
26. Cukras, C.; Wiley, H.E.; Jeffrey, B.G.; Sen, H.N.; Turriff, A.; Zeng, Y.; Vijayasarathy, C.; Marangoni, D.; Ziccardi, L.; Kjellstrom, S.; et al. Retinal AAV8-RS1 Gene Therapy for X-Linked Retinoschisis: Initial Findings from a Phase I/IIa Trial by Intravitreal Delivery. *Mol. Ther.* **2018**, *26*, 2282–2294. [CrossRef] [PubMed]
27. Pennesi, M.E.; Yang, P.; Birch, D.G.; Weng, C.Y.; Moore, A.T.; Iannaccone, A.; Comander, J.I.; Jayasundera, T.; Chulay, J. Intravitreal Delivery of rAAV2tYF-CB-hRS1 Vector for Gene Augmentation Therapy in Patients with X-Linked Retinoschisis: 1-Year Clinical Results. *Ophthalmol. Retin.* **2022**, *6*, 1130–1144. [CrossRef]
28. Scruggs, B.A.; Bhattarai, S.; Helms, M.; Cherascu, I.; Salesevic, A.; Stalter, E.; Laird, J.; Baker, S.A.; Drack, A.V. AAV2/4-RS1 gene therapy in the retinoschisin knockout mouse model of X-linked retinoschisis. *PLoS ONE* **2022**, *17*, e0276298. [CrossRef]
29. Sahel, J.-A.; Boulanger-Scemama, E.; Pagot, C.; Arleo, A.; Galluppi, F.; Martel, J.N.; Esposti, S.D.; Delaux, A.; de Saint Aubert, J.-B.; de Montleau, C.; et al. Partial recovery of visual function in a blind patient after optogenetic therapy. *Nat. Med.* **2021**, *27*, 1223–1229. [CrossRef]
30. Gaub, B.M.; Berry, M.H.; Holt, A.E.; Reiner, A.; Kienzler, M.A.; Dolgova, N.; Nikonov, S.; Aguirre, G.D.; Beltran, W.A.; Flannery, J.G.; et al. Restoration of visual function by expression of a light-gated mammalian ion channel in retinal ganglion cells or ON-bipolar cells. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, E5574–E5583. [CrossRef]
31. Gauvain, G.; Akolkar, H.; Chaffiol, A.; Arcizet, F.; Khoei, M.A.; Desrosiers, M.; Jaillard, C.; Caplette, R.; Marre, O.; Bertin, S.; et al. Optogenetic therapy: High spatiotemporal resolution and pattern discrimination compatible with vision restoration in non-human primates. *Commun. Biol.* **2021**, *4*, 125. [CrossRef]
32. Lam, B.L.; Zak, V.; Gonzalez, V.H.; Gregori, N.Z.; Chavala, S.H.; Batabyal, S.; Carlson, M.; Kim, S.; Ayyagari, A.; Chang, J.; et al. Safety and efficacy of MCO-010 optogenetic therapy in patients with Stargardt disease in USA (STARLIGHT): An open-label multi-center Ph2 trial. *eClinicalMedicine* **2025**, *87*, 103430. [CrossRef] [PubMed]
33. Duncan, J.L.; Branham, K.; Birch, D.G.; Daiger, S.P.; Ferris, F.L., III; Fishman, G.A.; Heon, E.; Hufnagel, R.B.; Pennesi, M.E.; Sahel, J.-A.; et al. Guidelines on Clinical Assessment of Patients with Inherited Retinal Degenerations—2022. Available online: https://www.aao.org/education/clinical-statement/guidelines-on-clinical-assessment-of-patients-with (accessed on 5 September 2025).

34. Lyons, B. The First Targeted Gene Therapy Approved in North America: Luxturna Faculty of Medicine, Centre for Blood Research: The University of British Columbia. 2018. Available online: https://cbr.ubc.ca/the-first-targeted-gene-therapy-approved-in-north-america-luxturna/ (accessed on 5 September 2025).
35. Burke, W.; Parens, E.; Chung, W.K.; Berger, S.M.; Appelbaum, P.S. The Challenge of Genetic Variants of Uncertain Clinical Significance: A Narrative Review. *Ann. Intern. Med.* **2022**, *175*, 994–1000. [CrossRef]
36. Botto, C.; Rucli, M.; Tekinsoy, M.D.; Pulman, J.; Sahel, J.A.; Dalkara, D. Early and late stage gene therapy interventions for inherited retinal degenerations. *Prog. Retin. Eye Res.* **2022**, *86*, 100975. [CrossRef]
37. Murthy, R.K.; Haji, S.; Sambhav, K.; Grover, S.; Chalam, K.V. Clinical applications of spectral domain optical coherence tomography in retinal diseases. *Biomed. J.* **2016**, *39*, 107–120. [CrossRef]
38. Strauss, R.W.; Kong, X.; Ho, A.; Jha, A.; West, S.; Ip, M.; Bernstein, P.S.; Birch, D.G.; Cideciyan, A.V.; Michaelides, M.; et al. Progression of Stargardt Disease as Determined by Fundus Autofluorescence Over a 12-Month Period: ProgStar Report No. 11. *JAMA Ophthalmol.* **2019**, *137*, 1134–1145. [CrossRef]
39. Health CAfDaTi. Clinical Review Report: Voretigene Neparvovec (Luxturna): (Novartis Pharmaceuticals Canada Inc.): Indication: Vision Loss, Inherited Retinal Dystrophy [Internet]: National Library of Medicine. 2021. Available online: https://www.ncbi.nlm.nih.gov/books/NBK569037/ (accessed on 5 September 2025).
40. Open-Label, Single Ascending Dose Study to Evaluate the Safety, Tolerability, and Efficacy of EDIT-101 in Adult and Pediatric Participants with Leber Congenital Amaurosis Type 10 (LCA10), with Centrosomal Protein 290 (CEP290)-Related Retinal Degeneration Caused by a Compound Heterozygous or Homozygous Mutation Involving c.2991+1655A>G in Intron 26 (IVS26) of the CEP290 Gene ("LCA10-IVS26") [Internet]. 2018. Available online: https://clinicaltrials.gov/study/NCT03872479 (accessed on 5 September 2025).
41. Deng, C.; Zhao, P.Y.; Branham, K.; Schlegel, D.; Fahim, A.T.; Jayasundera, T.K.; Khan, N.; Besirli, C.G. Real-world outcomes of voretigene neparvovec treatment in pediatric patients with RPE65-associated Leber congenital amaurosis. *Graefes Arch. Clin. Exp. Ophthalmol.* **2022**, *260*, 1543–1550. [CrossRef]
42. Darrow, J.J. Luxturna: FDA documents reveal the value of a costly gene therapy. *Drug Discov. Today* **2019**, *24*, 949–954. [CrossRef]
43. Hitch, J.; Denee, T.; Brassel, S.; Lee, J.; Michaelides, M.; Petersen, J.; Alulis, S.; Steuten, L. Challenges in Value Assessment for One-Time Gene Therapies for Inherited Retinal Diseases: Are We Turning a Blind Eye? *Value Health* **2025**, *28*, 116–124. [CrossRef] [PubMed]
44. Kessel, L.; Kjellberg, J.; Ibsen, R.; Rasmussen, A.; Nissen, K.R.; la Cour, M. Longitudinal analysis of health care costs in patients with childhood onset inherited retinal dystrophies compared to healthy controls. *BMC Ophthalmol.* **2022**, *22*, 466. [CrossRef]
45. Schofield, D.; Kraindler, J.; Tan, O.; Shrestha, R.N.; West, S.; Hart, N.; Tan, L.; Ma, A.; Grigg, J.R.; Jamieson, R.V. The health care and societal costs of inherited retinal diseases in Australia: A microsimulation modelling study. *Med. J. Aust.* **2023**, *219*, 70–76. [CrossRef] [PubMed]
46. Chay, J.; Tang, R.W.C.; Tan, T.E.; Chan, C.M.; Mathur, R.; Lee, B.J.H.; Chan, H.H.; Sim, S.; Farooqui, S.; Teo, K.Y.C.; et al. The economic burden of inherited retinal disease in Singapore: A prevalence-based cost-of-illness study. *Eye* **2023**, *37*, 3827–3833. [CrossRef] [PubMed]
47. Blindness, F.F. Inherited Retinal Diseases Cost the US and Canadian Economies up to $33 Billion Annually. Available online: https://www.fightingblindness.org/news/inherited-retinal-diseases-cost-the-us-and-canadian-economies-up-to-33-billion-annually-201?gad_source=1&gad_campaignid=23197030064&gbraid=0AAAAAD_ZajF9_BNSx0y7m_VPLhXNs43jp&gclid=Cj0KCQjwve7NBhC-ARIsALZy9HV_cVma2SbCBAc8HFsToY2Ne3u41mbVy50TMGazCZ_TwAl3U6y7sP8aAlPHEALw_wcB (accessed on 5 September 2025).
48. Ng, Q.X.; Ong, C.; Yaow, C.Y.L.; Chan, H.W.; Thumboo, J.; Wang, Y.; Koh, G.C.H. Cost-of-illness studies of inherited retinal diseases: A systematic review. *Orphanet J. Rare Dis.* **2024**, *19*, 93. [CrossRef]
49. Marques, J.P.; Ferreira, N.; Moreno, N.; Marta, A.; Vaz-Pereira, S.; Estrela-Silva, S.; Costa, J.; Cardoso, A.R.; Neves, P.; Duarte, L.; et al. Current management of inherited retinal degenerations in Portugal (IRD-PT survey). *Sci. Rep.* **2024**, *14*, 21473. [CrossRef] [PubMed]
50. Simonelli, F.; Sodi, A.; Falsini, B.; Bacci, G.; Iarossi, G.; Di Iorio, V.; Giorgio, D.; Placidi, G.; Andrao, A.; Reale, L.; et al. Care Pathway of RPE65-Related Inherited Retinal Disorders from Early Symptoms to Genetic Counseling: A Multicenter Narrative Medicine Project in Italy. *Clin. Ophthalmol.* **2021**, *15*, 4591–4605. [CrossRef] [PubMed]
51. Kherani, I.Z.; Andrews, C.; Pereira, J.A.; Moniz, L.S.; Qian, C.X. Impact of inherited retinal diseases on Canadian patients and families: A mixed-methods study. *Can. J. Ophthalmol.* **2023**, *58*, 532–538. [CrossRef]
52. Ail, D.; Malki, H.; Zin, E.A.; Dalkara, D. Adeno-Associated Virus (AAV)—Based Gene Therapies for Retinal Diseases: Where are We? *Appl. Clin. Genet.* **2023**, *16*, 111–130. [CrossRef]
53. Yang, P.; Mustafi, D.; Pepple, K.L. Immunology of Retinitis Pigmentosa and Gene Therapy-Associated Uveitis. *Cold Spring Harb. Perspect. Med.* **2024**, *14*, a041305. [CrossRef]

54. Hufnagel, R.B.; Ahmed, Z.M.; Corrêa, Z.M.; Sisk, R.A. Gene therapy for Leber congenital amaurosis: Advances and future directions. *Graefes Arch. Clin. Exp. Ophthalmol.* **2012**, *250*, 1117–1128. [CrossRef] [PubMed]
55. Colella, P.; Auricchio, A. Gene therapy of inherited retinopathies: A long and successful road from viral vectors to patients. *Hum. Gene Ther.* **2012**, *23*, 796–807. [CrossRef]
56. Liu, Y.; Zong, X.; Cao, W.; Zhang, W.; Zhang, N.; Yang, N. Gene Therapy for Retinitis Pigmentosa: Current Challenges and New Progress. *Biomolecules* **2024**, *14*, 903. [CrossRef]
57. Mack, H.G.; Britten-Jones, A.C.; McGuinness, M.B.; Chen, F.K.; Grigg, J.R.; Jamieson, R.V.; Edwards, T.L.; De Roach, J.; O'Hare, F.; Martin, K.R.; et al. Survey of perspectives of people with inherited retinal diseases on ocular gene therapy in Australia. *Gene Ther.* **2023**, *30*, 336–346. [CrossRef]
58. Britten-Jones, A.C.; Schultz, J.; Mack, H.G.; Kearns, L.S.; Huq, A.J.; Ruddle, J.B.; Mackey, D.A.; Hewitt, A.W.; Edwards, T.L.; Ayton, L.N. Patient experiences and perceived value of genetic testing in inherited retinal diseases: A cross-sectional survey. *Sci. Rep.* **2024**, *14*, 5403. [CrossRef]
59. Britten-Jones, A.C.; Mack, H.G.; Vincent, A.L.; Hill, L.J.; Edwards, T.L.; Ayton, L.N. Genetic testing and gene therapy in retinal diseases: Knowledge and perceptions of optometrists in Australia and New Zealand. *Clin. Genet.* **2024**, *105*, 34–43. [CrossRef]
60. Jinek, M.; Chylinski, K.; Fonfara, I.; Hauer, M.; Doudna, J.A.; Charpentier, E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* **2012**, *337*, 816–821. [CrossRef]
61. Maeder, M.L.; Stefanidakis, M.; Wilson, C.J.; Baral, R.; Barrera, L.A.; Bounoutas, G.S.; Bumcrot, D.; Chao, H.; Ciulla, D.M.; DaSilva, J.A.; et al. Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10. *Nat. Med.* **2019**, *25*, 229–233. [CrossRef]
62. Pulman, J.; Sahel, J.A.; Dalkara, D. New Editing Tools for Gene Therapy in Inherited Retinal Dystrophies. *Cris. J.* **2022**, *5*, 377–388. [CrossRef] [PubMed]
63. Quinn, J.; Musa, A.; Kantor, A.; McClements, M.E.; Cehajic-Kapetanovic, J.; MacLaren, R.E.; Xue, K. Genome-Editing Strategies for Treating Human Retinal Degenerations. *Hum. Gene Ther.* **2021**, *32*, 247–259. [CrossRef]
64. Park, S.H.; Bao, G. CRISPR/Cas9 gene editing for curing sickle cell disease. *Transfus. Apher. Sci.* **2021**, *60*, 103060. [CrossRef]
65. Yan, A.L.; Du, S.W.; Palczewski, K. Genome editing, a superior therapy for inherited retinal diseases. *Vision. Res.* **2023**, *206*, 108192. [CrossRef] [PubMed]
66. Dulla, K.; Slijkerman, R.; van Diepen, H.C.; Albert, S.; Dona, M.; Beumer, W.; Turunen, J.J.; Chan, H.L.; Schulkens, I.A.; Vorthoren, L.; et al. Antisense oligonucleotide-based treatment of retinitis pigmentosa caused by USH2A exon 13 mutations. *Mol. Ther.* **2021**, *29*, 2441–2455. [CrossRef]
67. Garanto, A.; Chung, D.C.; Duijkers, L.; Corral-Serrano, J.C.; Messchaert, M.; Xiao, R.; Bennett, J.; Vandenberghe, L.H.; Collin, R.W. In vitro and in vivo rescue of aberrant splicing in CEP290-associated LCA by antisense oligonucleotide delivery. *Hum. Mol. Genet.* **2016**, *25*, 2552–2563. [CrossRef] [PubMed]
68. Nikonov, S.; Aravand, P.; Lyubarsky, A.; Nikonov, R.; Luo, A.J.; Wei, Z.; Maguire, A.M.; Phelps, N.T.; Shpylchak, I.; Willett, K.; et al. Restoration of Vision and Retinal Responses After Adeno-Associated Virus-Mediated Optogenetic Therapy in Blind Dogs. *Transl. Vis. Sci. Technol.* **2022**, *11*, 24. [CrossRef] [PubMed]
69. Liang, Q.; Cheng, X.; Wang, J.; Owen, L.; Shakoor, A.; Lillvis, J.L.; Zhang, C.; Farkas, M.; Kim, I.K.; Li, Y.; et al. A multi-omics atlas of the human retina at single-cell resolution. *Cell Genom.* **2023**, *3*, 100298. [CrossRef] [PubMed]
70. Li, X.; Cai, S.; He, Z.; Reilly, J.; Zeng, Z.; Strang, N.; Shu, X. Metabolomics in Retinal Diseases: An Update. *Biology* **2021**, *10*, 944. [CrossRef]
71. Sobh, M.; Lagali, P.S.; Ghiasi, M.; Montroy, J.; Dollin, M.; Hurley, B.; Leonard, B.C.; Dimopoulos, I.; Lafreniere, M.; Fergusson, D.A.; et al. Safety and Efficacy of Adeno-Associated Viral Gene Therapy in Patients With Retinal Degeneration: A Systematic Review and Meta-Analysis. *Transl. Vis. Sci. Technol.* **2023**, *12*, 24. [CrossRef] [PubMed]

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Genomic Medicine Among Ophthalmologists: Knowledge, Current Practice, and Barriers

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Abstract

Background/Objectives: To assess the knowledge, attitudes, and practices of ophthalmologists in Saudi Arabia towards genomic medicine and genetic testing, in light of the growing significance of genomics in ophthalmology and the national transition towards precision medicine. **Methods**: A cross-sectional, questionnaire-based survey was conducted among ophthalmologists, including consultants, specialists, fellows, and residents, across Saudi Arabia. The questionnaire included four domains: demographics, knowledge of genomic principles and gene therapy, self-rated confidence in genetic tasks (scored 1–10), and attitudes toward genetic testing. Data were analyzed using descriptive and inferential statistics, with subgroup comparisons performed using chi-square tests and *t*-tests/ANOVA. **Results**: A total of 115 ophthalmologists participated (46% male, 54% female; mean age 34 years; mean post-board experience 4 years). Most were consultants (40%) and practiced in Riyadh (52%). Knowledge was variable: 92% correctly identified human chromosome count, and 99% recognized autosomal recessive inheritance, but only 9% answered DNA base-pairing correctly, and 54% recognized mitochondrial inheritance. Confidence was highest for referral to specialists (mean 7.3/10) and lowest for test selection and counseling (4.7/10). The internet was the primary knowledge source among our sample (65%). The majority of individuals had positive attitudes towards genomic medicine: 90% believed testing was beneficial, 89% considered it enhanced health outcomes, and 89% indicated they would undergo testing themselves. On the other hand, 77% indicated difficulty in access, 91% strongly concurred on the significance of privacy and confidentiality, and more than half expressed concerns regarding misuse and bias. **Conclusions**: Ophthalmologists in Saudi Arabia acknowledge the importance of genetics. Yet, there are substantial gaps in knowledge and familiarity with genomic medicine and genetic testing. To overcome these challenges, it is essential to integrate genetics into ophthalmology curricula.

Keywords: knowledge; attitude; practices; genomic medicine; ophthalmologists

1. Introduction

Genomic medicine is an evolving part of healthcare today and integrates genetic and molecular data into clinical decision-making to improve disease prevention, diagnosis, and treatment. In ophthalmology, genomic medicine improves diagnostic accuracy and facilitates the adoption of a personalized approach for numerous diseases that have a genetic basis, such as retinal dystrophies, congenital glaucoma, and hereditary corneal disorders. Despite these developments in the medical field, research from different countries continues to show significant gaps in the understanding of this field amongst healthcare professionals [1–4]. Such gaps in understanding genomic medicine might affect patient care through delayed referrals to ophthalmic genetic services, delayed genetic testing, and absent or deficient counseling for family members against consanguinity. Surveys of medical students and physicians in the Arab countries revealed predominantly favorable opinions towards genomic medicine, although they highlighted a lack of formal education and practical training. In these studies, healthcare professionals recognized insufficient training and interpretative abilities as significant obstacles in integrating genomics in their practice [5]. Research in Saudi Arabia has demonstrated similar observations; pharmacists and physicians have shown positive attitudes about genetic medicine, but exhibited confusion regarding the application of test results in clinical practice [6,7]. In ophthalmology, genetic testing and gene-based therapies are rapidly evolving, making it critical that physicians in these specialties have a good background and understanding of this rapidly evolving field. Ophthalmologists need to be aware that gene therapy is transforming the treatment of various ophthalmic conditions by addressing the root causes of debilitating diseases like inherited retinal dystrophies [8]. In addition, ophthalmology is one of the leading specialties in the clinical applications of gene therapy, and appropriate referral by ophthalmologists for genetic testing directly impacts eligibility for emerging treatments. In a country with high rates of consanguinity like Saudi Arabia, the accessibility to ophthalmic genetic services and the availability of genetic testing with meaningful interpretation might reflect positively on medical care for affected patients, as well as for other family members through early prevention and premarital genetic counseling. Ethical, legal, and societal issues—such as the proper use of genetic information, possible discrimination, and patient confidentiality—however, remain important considerations [9,10]. In the era of personalised medicine, identification of gaps in the knowledge and understanding of genomic medicine among physicians and ophthalmologists facilitates the transition to personalised medicine. The availability of more genomic information and molecular mechanisms of inherited diseases leads to a better understanding of disease and higher-quality care provided to patients. Improving the practices and applications of genomic medicine among ophthalmologists through proper identification of patients, utilization of genetic testing to yield comprehensive genomic information, and appropriate patient referral represent integral steps towards personalised patient care. Our study aims to evaluate the knowledge, attitudes, and practices of ophthalmologists in Saudi Arabia towards genomic medicine and genetic testing, given the evolving significance of genomics in ocular care. It also assesses demographic factors, understanding of genetics and gene therapy, self-reported trust in genetic counseling and testing, and perspectives on the clinical and ethical aspects of genetic medicine.

2. Materials and Methods

This study was a cross-sectional, questionnaire-based study among ophthalmologists in Saudi Arabia. Eligible participants were ophthalmology consultants, specialists, fellows, and residents. The survey was distributed electronically to ensure accessibility across all regions of the Kingdom. The study was approved by IRB at KKESH as the Saudi Ophthal-

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mology Society (SOS) has no IRB. The SOS has ophthalmologists' contacts, not the collected data, because data collection was performed based on the response from ophthalmologists in this study. The questionnaire (Table S1) was modified from recognized questionnaires used to evaluate knowledge, attitudes, and practices in genomic medicine and tailored for the ophthalmology domain. It consisted of four domains: (1) demographics, (2) understanding of genetic principles, inheritance mechanisms, and gene therapy, (3) self-rated confidence in performing genomics-related tasks such as family history assessment, test interpretation, and counseling, and (4) attitudes and perceptions regarding genomic testing, its availability, benefits, and ethical concerns. To test the understanding of genetic principles, inheritance mechanisms, and gene therapy, questions were modified from a survey that was provided for healthcare professionals [11]. To test self-rated confidence to perform genomics-related tasks, questions were modified from a survey that was provided for primary care physicians [12]. Both sets of questions were modified to more appropriately address ophthalmologists. The face validity of the survey was assessed by ten ophthalmologists to ensure the clarity and the premise of each question. The survey was then piloted for content, design, readability, and comprehension by ten different ophthalmologists. The required amendments were made based on the feedback to develop the final survey. The survey was distributed using professional ophthalmology email lists and contacts between June and August 2025. All ophthalmologists who had active memberships in the Saudi Ophthalmology Society (SOS) were invited to participate in the study. Ophthalmologists who did not have active SOS memberships were excluded. The total number of ophthalmologists who were invited was 429 ophthalmologists. Informed consent was obtained electronically prior to survey completion. The data extraction from the responses was performed electronically through predefined variables and was then transferred to the IBM Statistical Package for Social Sciences (SPSS). The SPSS version 30.0.0 was used to perform the statistical analysis. Descriptive statistics were generated for categorical variables (frequencies, percentages) and continuous variables (means, standard deviations, medians, ranges). Knowledge questions were analyzed for correctness; confidence scores are summarized as mean $\pm$ SD. Attitudinal responses are reported as proportions.

3. Results

A total of 115 ophthalmologists participated in our study, and the response rate was 26.8%. Of these, 53 (46.1%) were male, and 62 (53.9%) were female (Figure 1). The mean age of respondents was 34.4 years (SD $\pm$ 7.6; range: 26–60), with a median of 32 years. The mean post-residency clinical experience was 4.3 years (SD $\pm$ 5.6; range: 0–30), with a median of 2 years. In terms of professional grade, 45 respondents (39.8%) were consultants, 11 (9.7%) specialists, 35 (31.0%) fellows, and 24 (21.2%) residents (Figure 2). The majority of participants had their ophthalmology practice based in Riyadh (52%), followed by Jeddah (9.6%), Alkhobar (7.8%), Dammam (7.0%), and Dhahran (7.0%).

Knowledge questions revealed variable levels of accuracy. Almost all participants correctly identified that humans do not have 50 chromosomes (92%), and 99% recognized misconceptions in statements about autosomal recessive inheritance. In addition, 86% recognized misconceptions in statements about autosomal dominant inheritance. Similarly, 85% understood that not all genetic variants cause disease. Importantly, 85% of ophthalmologists confirmed that gene therapy is no longer confined to research purposes only (85%). However, only 9% correctly answered the DNA base pairing question, and 54% recognized the maternal inheritance pattern of mitochondrial diseases. Sources of information were primarily through the internet (65%), followed by books (14%) and medical school (12%).

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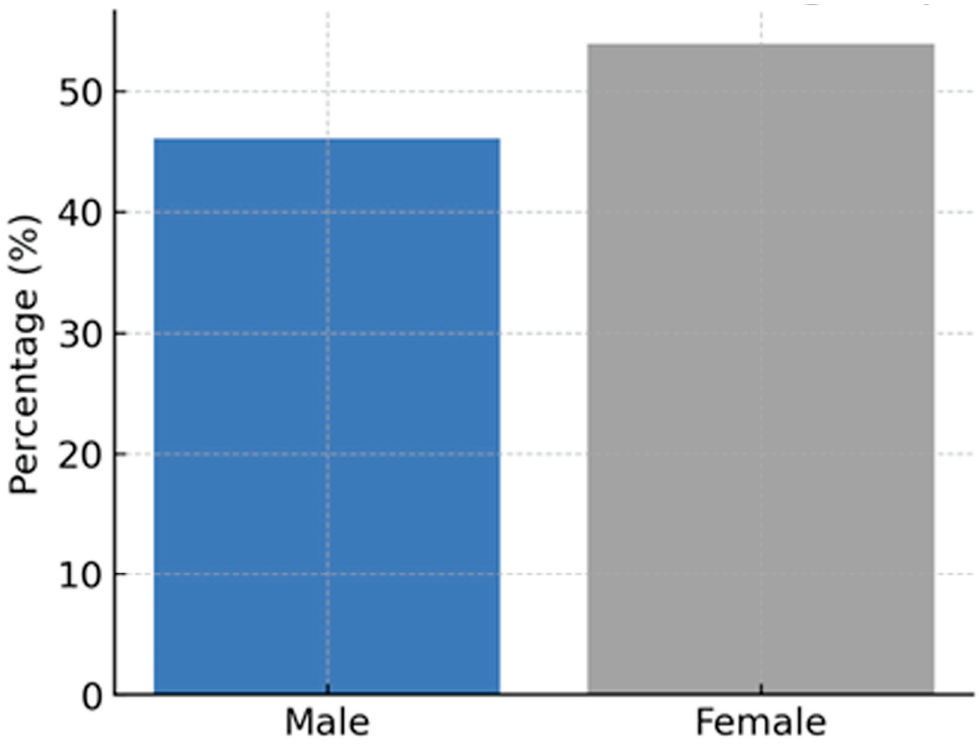

Figure 1. Gender distribution among respondents.

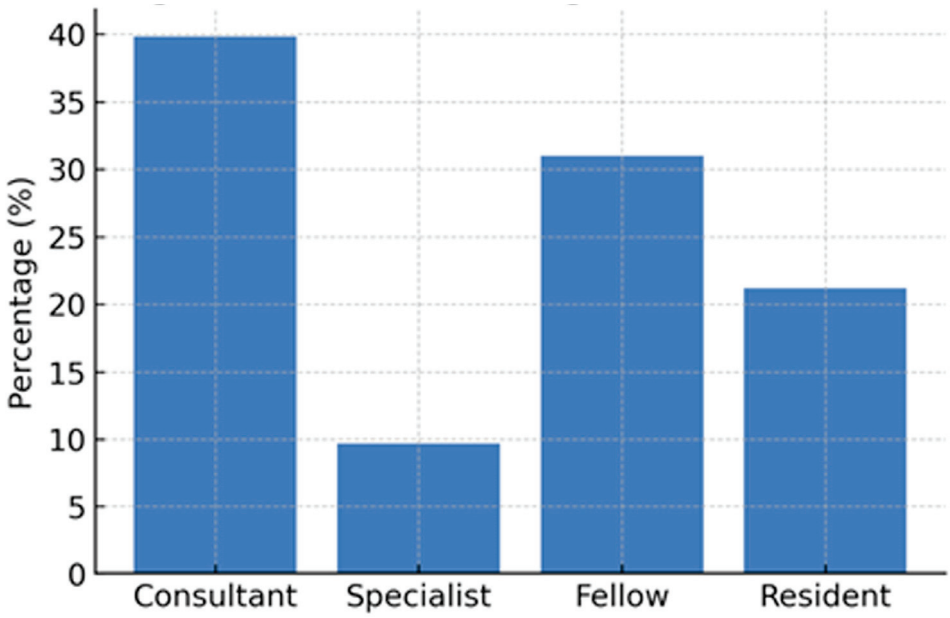

Figure 2. Professional grades of ophthalmologists who responded to the survey.

Confidence scores indicated moderate overall confidence. The highest mean scores were reported for referral to specialists (mean 7.3 ± 2.3) and obtaining family history (mean 6.6 ± 2.3). Clinical evaluation scored moderately (mean 5.5 ± 2.3). In contrast, confidence in deciding appropriate genetic tests (mean 4.7 ± 2.8), discussing prenatal diagnosis (mean 4.8 ± 2.8), and providing detailed counselling was low. This pattern highlights the reliance on referral networks rather than independent genetic decision-making).

The majority of participants considered genetic testing to be beneficial (90%) and reported that it has a favorable impact on health and well-being (89%). A substantial proportion (89%) were in favor of having genetic evaluations if recommended. Nonethe-

less, 77% believed that testing is not readily accessible to the public. Ninety-one percent displayed great agreement with the importance of confidentiality and privacy. Forty-nine percent of individuals expressed concern regarding the potential misuse of genetic data, while 51% indicated apprehension about the possibility of genetic data resulting in discrimination in employment, insurance, or healthcare. Most participants (89%) predicted that genetic testing would become more prevalent in the future. Figure 3 illustrates that individuals exhibited the highest confidence in recommending someone to an expert, while they demonstrated the lowest confidence in selecting a test or providing advice. Figure 4 illustrates that the majority of individuals perceived testing as beneficial; nonetheless, numerous concerns around privacy, accessibility, and potential misuse were prevalent.

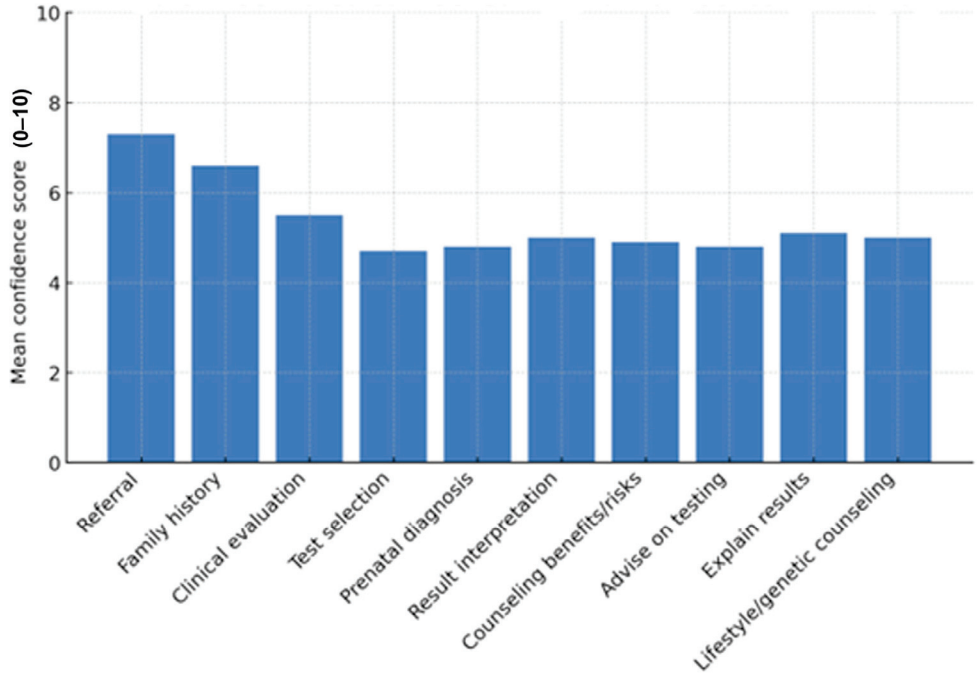

Figure 3. Self-rated confidence in performing clinical tasks that are related to the genomic medicine practice.

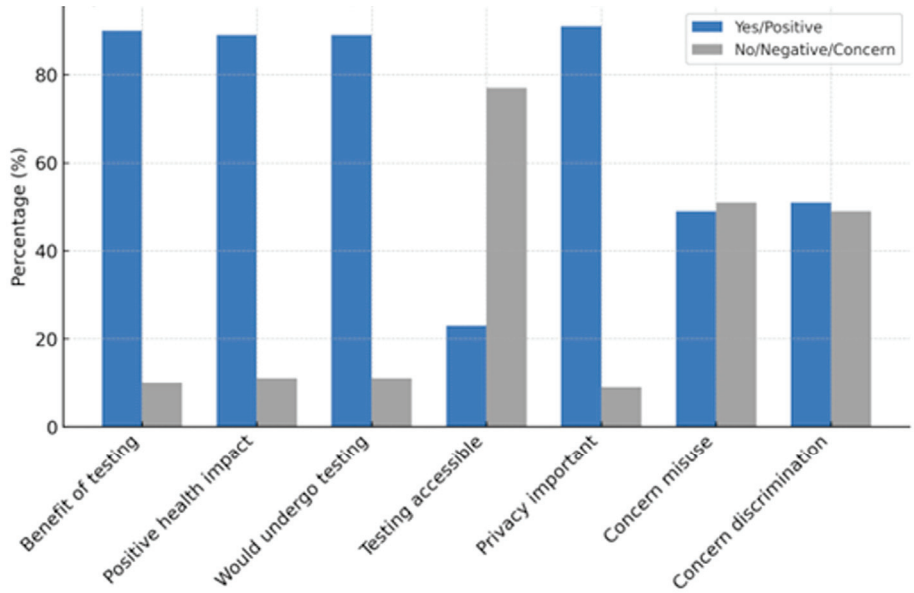

Figure 4. Attitudes and perceptions of ophthalmologists towards genetic testing.

4. Discussion

This study explored the knowledge, attitudes, and practices of ophthalmologists in Saudi Arabia regarding genomic medicine and genetic testing. To the best of our knowledge, this is the first study to address the gap in understanding genomic medicine and genetic testing in the field of ophthalmology in our region. Based on our survey analysis, our findings indicate significant deficiencies in knowledge, limited confidence in clinical application, and generally positive attitudes toward genetic ophthalmic illnesses. These results, when applied in the context of regional and global data across various medical specialties, suggest the need for comprehensive training, improved infrastructure, and amendments to medical school curricula to enable the effective integration of genetics into ophthalmic service and care.

Despite the advancements in the Human Genome Project [2] and the expanding global understanding and application of genomic medicine [1], our findings reveal ongoing knowledge gaps among ophthalmologists. Participants demonstrated a good understanding of inheritance patterns; yet, some errors related to molecular genetics were found, such as DNA base pairing and mitochondrial inheritance mechanisms. These results support the previous findings highlighting similar deficiencies in knowledge among medical students and pharmacists in Saudi Arabia, as well as physicians who demonstrated uncertainty in the application of pharmacogenomics despite acknowledging its clinical relevance [6,7]. These results also show that while ophthalmologists retained certain background information about human genetics, they were not exposed to more sophisticated material in molecular genetics and special types of inheritance. As only a minority (12%) retained their information from medical school, more in-depth exposure to genomic medicine during medical school, and more importantly, at later stages of their careers, is needed to expand the knowledge of ophthalmologists about genomic medicine.

The consequences of deficient knowledge about genomic medicine are particularly relevant in ophthalmology more than in other specialties. The abundance of inherited retinal diseases, which are associated with an increasing number of genetic variants in disease-causing genes [3], makes the knowledge about genomic medicine and ophthalmic genetics essential for appropriate diagnosis and referrals. The emergence of gene-based therapies further emphasizes the critical importance of early diagnosis and referrals for centers that provide genetic therapy trials.

Notably, our findings were in alignment with international studies demonstrating that non-genetic specialists in other medical specialties—including cardiologists, oncologists, and neurologists—report inadequate training and poor preparedness for genomic applications in clinical practice [13–15]. A comprehensive survey in Australia confirmed that while clinicians recognize genomic relevance, most feel unprepared to implement these tools without substantial additional training [15]. The low correct response rate for the DNA base pairing (9%) in our study, in contrast to the high correct responses for the numbers of chromosomes and the modes of inheritance, reflects the lack of depth in knowledge about genomic medicine despite the good understanding of the general concepts of the subject.

Confidence levels among our participants varied significantly across different genomic medicine applications. Participants expressed the highest confidence in referring patients to genetics specialists but expressed lower confidence in ordering tests independently, interpreting results, and providing genetic counseling. Similar patterns of resorting to referral pathways rather than developing one's own genomic competencies have been widely reported in the literature [15–17]. Similar findings were found among cardiology and oncology studies, where clinicians admitted to avoiding direct genomic decision-making due to a lack of confidence [14]. These patterns have been observed throughout Middle Eastern and Arab countries, where clinicians might lack the appropriate exposure

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to genomic medicine during training and require improvements in genetic services [6,16]. Although referring patients to genetic specialists might be an appropriate decision when dealing with patients with inherited eye diseases, the referral to ophthalmic geneticists might not be easy for some patients who resort to performing genetic testing in the most accessible facility. Ophthalmologists who are confident in interpreting genetic testing might make a difference in the management of these patients through careful monitoring of patients with syndromic or nonsyndromic pathological myopia or appropriate referrals for other specialties for syndromes that involve multiple organs, like Usher syndrome and Bardet–Biedl syndrome [18]. The National Genomic Initiative might need to address these gaps in knowledge and confidence through continuous medical education activities and workshops for ophthalmologists.

Our results highlight that informal educational resources, which include online materials and presentations, served as primary sources of genomic knowledge. These findings indicate broader learning patterns among health care professionals that extend beyond structured, comprehensive education [5–7,16]. Such reliance on informal learning might contribute to incomplete and insufficient understanding and reinforces the critical need for specialty-specific genomic curricula.

The majority of participating ophthalmologists demonstrated positive attitudes toward genetic testing, with nearly 90% expressing willingness to undergo testing themselves if clinically advised. This optimism is supported by the recent advances and the introduction of precision medicine and individualized patient care [1,4,19]. However, participants identified substantial barriers to implementation, including limited service availability, high costs, and insufficient institutional readiness. These concerns align with issues raised throughout the Middle East [6] and persist even in Western countries where limitations in infrastructure and challenges in reimbursement remain significant obstacles [11,19].

Participants expressed significant concerns regarding data confidentiality, genetic discrimination, and the potential for misuse of genetic information. These ethical challenges were not confined to Saudi Arabia, as similar apprehensions were expressed across Canada, the United States, and Europe [10,19]. DuBois et al. specifically reported that both patients and clinicians emphasize the fundamental importance of trust, privacy safeguards, and comprehensive informed consent in the implementation of genomic medicine [19]. Knoppers and Beauvais similarly argued that ethical considerations are inseparable from successful clinical integration of genomic medicine [9].

Our findings suggest that ophthalmologists in Saudi Arabia are not yet adequately prepared for the integration of genomic medicine into their routine practice, despite recognizing its substantial clinical value. This readiness gap is particularly concerning given that precision medicine has been designated as a national health priority, with Saudi Arabia making significant investments in genomics infrastructure and workforce development [17]. For ophthalmology specifically, strengthening genomic literacy is essential to support effective integration of genomic medicine in clinical practice and optimal uptake of emerging therapies for inherited eye diseases today and in the future.

This study is the first study assessing the genetic competencies of ophthalmologists in Saudi Arabia, which provides specialty-specific evidence to the regional literature. In addition, it provides a foundation for educational and policy reforms that could enhance public preparedness for genomics. Limitations of this study include dependence on self-reported data, which may inadequately represent actual competencies, and the cross-sectional design, which restricts causal inference. About half of the study participants were from Riyadh, and this might have resulted in overestimation or underestimation of knowledge and confidence. In addition, this geographical distribution might limit the generalizability of results and might have resulted in a sampling bias. The relatively small

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sample size and unknown response rate further affect representativeness. Additionally, the cross-sectional design might limit the causal inference. This design cannot determine whether increased training would directly improve confidence or practice.

5. Conclusions

This study demonstrates that while ophthalmologists in Saudi Arabia generally recognize the importance of genomic medicine and express positive attitudes toward its use, significant knowledge gaps, limited confidence in clinical application, and systemic barriers hinder its integration into practice. These findings mirror international trends and emphasize the urgent need for structured genomic education, improved infrastructure, and clear legislative frameworks to ensure safe and effective implementation. Strengthening genomic knowledge and confidence among ophthalmologists might be critical not only for accurate diagnosis and timely referral but also for enabling the successful adoption of emerging gene-based therapies and advancing precision medicine in Saudi Arabia. Concrete steps that support this direction include higher exposure for ophthalmic genetics among ophthalmology residents, establishing multidisciplinary clinics with genetic counselors, and developing national guidelines for genetic testing in ophthalmology.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jpm16050267/s1, Table S1: Knowledge, Attitude and Practices towards Genomic Medicine among Ophthalmologists in Saudi Arabia.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of King Khaled Eye Specialist Hospital (KKESH); reference: 45148-P. 3/11/2024.

Informed Consent Statement: Informed consent was obtained electronically from the participants after explaining the aims of the study.

Data Availability Statement: All the data presented in this manuscript are available on request.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

KAP knowledge, attitudes, and practices

References

1. Collins, F.S.; Morgan, M.; Patrinos, A. The Human Genome Project: Lessons from large-scale biology. *Science* **2003**, *300*, 286–290. [CrossRef]
2. Daiger, S.P.; Sullivan, L.S.; Bowne, S.J. Genes and mutations causing retinitis pigmentosa. *Clin. Genet.* **2013**, *84*, 132–141. [CrossRef]
3. Haga, S.B.; O'Daniel, J.M.; Tindall, G.M.; Lipkus, I.R.; Agans, R. Survey of US public attitudes toward pharmacogenetic testing. *Pharmacogenom. J.* **2012**, *12*, 197–204. [CrossRef]
4. Olwi, D.; Merdad, L.; Ramadan, E. Knowledge of Genetics and Attitudes toward Genetic Testing among College Students in Saudi Arabia. *Public Health Genom.* **2016**, *19*, 260–268. [CrossRef] [PubMed]

5. Alzoubi, A.; Kanaan, H.; Alhazaimeh, D.; Gharaibeh, S.; Mukattash, T.L.; Kheirallah, K. Knowledge, attitude, future expectations, and perceived barriers of medical students and physicians regarding pharmacogenomics in Jordan. *Int. J. Clin. Pract.* **2020**, *75*, e13658. [CrossRef]
6. Alrabiah, Z.; Syed, W.; Babelghaith, S.D.; Al Arifi, M.N. Clinical Knowledge, Attitude, and Perceptions of Community Pharmacists Towards Pharmacogenomics—A Cross-Sectional Study from Saudi Arabia. *Pharmacogenom. Pers. Med.* **2023**, *16*, 433–441. [CrossRef] [PubMed] [PubMed Central]
7. Alsanosi, S.M.; Al Eissa, M.M. Awareness of pharmacogenomic capabilities among physicians and pharmacists in Saudi Arabia within current clinical practice. *J. Umm Al-Qura Univ. Med. Sci.* **2025**, *11*, 1. [CrossRef]
8. AlEissa, M.M.; Alhawsawi, A.A.; Alonazi, R.; Magharbil, E.; Aljahdali, A.; AlBalawi, H.B.; Alali, N.M.; Hameed, S.; Abu-Amero, K.K.; Magliyah, M.S. Advances in Precision Therapeutics and Gene Therapy Applications for Retinal Diseases: Impact and Future Directions. *Genes* **2025**, *16*, 847. [CrossRef]
9. Knoppers, B.M.; Beauvais, M.J.S. Three decades of genetic privacy: A metaphoric journey. *Hum. Mol. Genet.* **2021**, *30*, R156–R160. [CrossRef] [PubMed] [PubMed Central]
10. Manolio, T.A.; Chisholm, R.L.; Ozenberger, B.; Roden, D.M.; Williams, M.S.; Wilson, R.; Bick, D.; Bottinger, E.P.; Brilliant, M.H.; Eng, C.; et al. Implementing genomic medicine in the clinic: The future is here. *Genet. Med.* **2013**, *15*, 258–267. [CrossRef]
11. AlRasheed, M.M.; AlAli, H.; Alsuwaid, A.F.; Khalaf, S.; Ata, S.I.; BinDhim, N.F.; Bakheet, D.; Khurshid, F.; Alhawassi, T.M. Gene Therapy Knowledge and Attitude Among Healthcare Professionals: A Cross-Sectional Study. *Front. Public Health* **2021**, *9*, 773175. [CrossRef] [PubMed] [PubMed Central]
12. Yu, M.W.C.; Fung, J.L.F.; Ng, A.P.P.; Li, Z.; Lan, W.; Chung, C.C.Y.; Li, Y.; Liu, Y.; Chung, B.H.Y.; Wong, W.C.W. Preparing for the genomic revolution: Attitudes, clinical practice, and training needs in delivering genetic counseling in primary care in Hong Kong and Shenzhen, China. *Mol. Genet. Genom. Med.* **2021**, *9*, e1702. [CrossRef] [PubMed] [PubMed Central]
13. Chow-White, P.A.; Ha, D.; Laskin, J. Knowledge, attitudes, and values among physicians working with clinical genomics: A survey of medical oncologists. *Hum. Resour. Health* **2017**, *15*, 42. [CrossRef]
14. Christensen, K.D.; Vassy, J.L.; Jamal, L.; Lehmann, L.S.; Slashinski, M.J.; Perry, D.L.; Robinson, J.; Blumenthal-Barby, J.; Feuerman, L.; Murray, M. Are physicians prepared for whole genome sequencing? A qualitative analysis. *Clin. Genet.* **2016**, *89*, 228–234. [CrossRef]
15. Haga, S.B.; Kim, E.; Myers, R.A.; Ginsburg, G.S. Primary care physicians' knowledge, attitudes, and experience with personal genetic testing. *J. Pers. Med.* **2019**, *9*, 29. [CrossRef] [PubMed]
16. Mawkili, W.A. The future of personalized medicine in Saudi Arabia: Opportunities and challenges. *Saudi Med. J.* **2025**, *46*, 19–25. [CrossRef]
17. AlEissa, M.M.; Alhawsawi, A.A.; Milibari, D.; Schatz, P.; AlBalawi, H.B.; Alali, N.M.; Abu-Amero, K.K.; Hameed, S.; Magliyah, M.S. Genetics and Clinical Findings Associated with Early-Onset Myopia and Retinal Detachment in Saudi Arabia. *Genes* **2025**, *16*, 848. [CrossRef] [PubMed]
18. DuBois, J.M.; Callier, S.L.; Garrison, N.A.; James, C.A.; Henderson, G.E. Attitudes and expectations toward genomics and precision medicine: Perspectives from medical professionals and patients. *BMC Med. Ethics* **2021**, *22*, 41.
19. Katsanis, S.H.; Katsanis, N. Molecular genetic testing and the future of clinical genomics. *Nat. Rev. Genet.* **2013**, *14*, 415–426. [CrossRef] [PubMed]

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Intravitreal Aflibercept for the Treatment of Diabetic Retinopathy Among Patients Who Completed PANORAMA: 1-Year Outcomes from the VOYAGE Extension Study

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Abstract: Background/Objectives: Evaluate outcomes and treatment patterns with 2 mg intravitreal aflibercept injection among patients who completed the phase 3 PANORAMA trial and enrolled in the VOYAGE (ClinicalTrials.gov identifier, NCT04708145; 12 January 2021) long-term extension study. **Methods**: During VOYAGE, patients were evaluated every 16 weeks and treated with 2 mg intravitreal aflibercept injection as needed depending on ophthalmoscopic examination findings. Those with no history of panretinal photocoagulation (PRP) received aflibercept if their clinician-determined diabetic retinopathy severity scale (DRSS) level was $\geq$47, corresponding to moderately severe non-proliferative diabetic retinopathy (NPDR). Patients with a history of PRP received aflibercept if active neovascularization was present. New or worsening diabetic retinopathy (DR) severity prompted more frequent treatment. **Results**: 320 patients (1 eye per patient) from 87 sites completed the PANORAMA trial. Of these, 41 patients (13% of PANORAMA completers) from 14 sites (16%) enrolled in VOYAGE after a mean interim period of 33.7 months, and 35 patients (85%) completed study visits through 1 year. At year 1 in VOYAGE, the mean number of anti-vascular endothelial growth factor (VEGF) injections increased from 1.1 per year during the interim period to 3.4 per year and was associated with stabilization or improvement in DRSS level in 81% (26/32) of patients. Mean best-corrected visual acuity (BCVA) remained relatively stable, and mean central subfield thickness (CST) improved by 24.4 μm to 269.5 μm through year 1 of VOYAGE. There were no unexpected safety events. **Conclusions**: Following a mean of 3 years of routine clinical care with associated declines

in DRSS level, CST, and BCVA, stabilization of DRSS level and BCVA with reductions in CST was achieved through year 1 of the VOYAGE extension study, with a concurrent increase in aflibercept dosing frequency.

Keywords: aflibercept; diabetic retinopathy; anti-VEGF; extension study

1. Introduction

Diabetic retinopathy (DR) remains a leading cause of global vision loss and is estimated to affect 9.6 million people in the United States alone [1]. Vision-threatening complications from DR include proliferative DR (PDR) and diabetic macular edema (DME) [2]. Diabetic retinopathy severity can be assessed using structured grading of fundus color images based on the Early Treatment Diabetic Retinopathy Study (ETDRS) severity scale [3]. There are 12 steps within the Diabetic Retinopathy Severity Scale (DRSS): levels 10 and 20 represent no DR and very mild DR, respectively; levels 35 and 43 represent mild and moderate non-proliferative DR (NPDR), respectively; levels 47 and 53 represent moderately severe and severe NPDR, respectively; and levels 60, 61, 65, 71, 75, 81, and 85 represent increasing severities of PDR [3]. A higher level within the NPDR portion of the ETDRS DRSS is highly predictive of progression to PDR [4], a finding confirmed in multiple recent prospective clinical trials [5,6].

Anti-vascular endothelial growth factor (VEGF) pharmacotherapy has demonstrated the ability to slow DR progression, reduce DME and PDR development, and improve DRSS levels in a meaningful proportion of patients [6,7]. The randomized, double-masked, sham-controlled 100-week, phase 3 PANORAMA (NCT02718326) trial evaluated 2 mg intravitreal aflibercept injection in patients with moderately severe to severe NPDR (DRSS levels 47 and 53) [6]. In PANORAMA, 402 patients (1 eye per patient) with best-corrected visual acuity (BCVA) of 20/40 or better were randomized to either sham, aflibercept every 16 weeks following 3 initial monthly doses and one 8-week interval (aflibercept 2q16), or aflibercept every 8 weeks following 5 initial monthly doses with pro re nata (PRN) dosing in year 2 if DRSS level was worse than 35 (aflibercept 2q8/PRN). Findings from PANORAMA revealed a significantly greater proportion of patients in the aflibercept 2q8/PRN and 2q16 groups experienced a $\geq$ 2-step DRSS level improvement from baseline at week 52 compared with those in the sham group (80% and 65% versus 15%, respectively), and these improvements were maintained through week 100. Most clinically relevant, fewer patients treated with aflibercept versus sham experienced development of PDR or DME through week 100.

For many patients, DR and its associated manifestations is a chronic disease, and long-term close monitoring and personalized care are needed to achieve optimal clinical outcomes [8–10]. However, data on long-term outcomes are limited among patients with NPDR without DME receiving anti-VEGF injections. The VOYAGE phase 4 open-label PANORAMA extension study was therefore conducted to evaluate the long-term outcomes of patients who completed PANORAMA after an intervening period of routine clinical care and help contribute to our understanding of the long-term efficacy and safety of anti-VEGF treatment among these patients.

2. Materials and Methods

VOYAGE (ClinicalTrials.gov identifier, NCT04708145; 12 January 2021) was a prospective, phase 4 open-label extension study which included patients who completed the preceding phase 3 PANORAMA trial. The study protocol was approved by the Advarra

Institutional Review Board and Ethics Committee at each participating site, and the study was performed in adherence with the principles of the Declaration of Helsinki and local regulations [11]. All patients provided written informed consent. Data from patients between the end of PANORAMA and VOYAGE enrollment (the interim period) were retrospectively collected to evaluate patterns of treatment and clinical outcomes during this phase. Collected data included demographic information, visit dates, original randomized groups in PANORAMA, anti-VEGF treatments, laser procedures such as panretinal photocoagulation (PRP), phakic status, BCVA, central subfield thickness (CST), DRSS level, and glycosylated hemoglobin (HbA1c) when available.

During VOYAGE, patients were treated with 2 mg intravitreal aflibercept injection PRN depending on ophthalmoscopic examination findings and investigator-determined DRSS level. Based on the presence or absence of prior PRP application at VOYAGE enrollment, patients were categorized into Group 1, eyes without PRP, or Group 2, eyes with PRP.

Patients with study eyes without PRP (Group 1) were evaluated every 16 weeks and treated with 2 mg intravitreal aflibercept injection if the clinician-determined DRSS level was 47 (moderately severe NPDR) or worse; patients with DRSS level < 47 were not treated. The interval between visits was decreased to every 8 weeks if DRSS level was $\geq$ 53, there was $\geq$2-step DRSS level worsening compared to the last protocol-scheduled 16-week visit, or if the patient developed active PDR. The patient could then be treated with aflibercept every 8 weeks until there was no active PDR and DRSS improved to the level observed at the visit before the patient began being seen at 8-week intervals.

Patients with study eyes with PRP (Group 2) were evaluated every 16 weeks and treated with 2 mg intravitreal aflibercept injection if the neovascular disease process was active and stable. If the neovascular process was determined to be inactive, no treatment was given. If new or worsening neovascular disease was present, patients were treated every 8 weeks until the process became stable or inactive, at which time the interval between visits increased to every 16 weeks.

If high-risk PDR (defined as neovascularization of the disc greater than 1/3 disc area or any neovascularization associated with vitreous or preretinal hemorrhage) developed, PRP therapy could be applied at the investigator's discretion, although PRP was not required, and investigators were allowed to use aflibercept monotherapy for PDR management. If vitreous or preretinal hemorrhage developed, aflibercept could have been given as often as every 4 weeks until hemorrhage improved and was no longer considered vision-threatening. Patients who developed clinically relevant DME (defined as CST > 320 μm on optical coherence tomography [OCT] or clinically worse than baseline with associated vision loss) could have been treated with aflibercept every 4 or 8 weeks at the investigator's discretion until the DME resolved.

At all visits, patients underwent ETDRS BCVA testing at 4 m, slit lamp and dilated ophthalmic examination, and spectral-domain OCT imaging. Ultra-widefield fundus photography was performed every 16 weeks and was used to determine DRSS levels and presence of center-involving DME by a central reading center (Doheny Image Reading Center, Los Angeles, CA, USA). Per the reading center protocol, eyes could be graded as DRSS level < 61 if there were no active PDR signs, regardless of the presence of PRP scars.

The objective of VOYAGE was to assess longitudinal DRSS level changes, and the primary efficacy endpoint was the proportion of patients achieving DRSS level of 43 (moderate NPDR) or better at 1 year. Secondary outcomes were the proportion of patients with stable, worsened, or improved DRSS level; mean and annualized aflibercept injection frequencies; mean change in ETDRS BCVA; mean change in CST; and incidence of ocular and systemic adverse events (AEs).

All analyses used observed data. The denominators for percentages varied depending on the total data available for that variable. Group comparisons were based on two-tailed *t*-tests, paired where applicable, or analysis of variance (ANOVA) for continuous variables, and chi-square tests for categorical variables. Statistical analyses were performed using Microsoft Excel 2016 (Microsoft Corp, Redmond, WA, USA) and GraphPad Prism version 10.6.0 for Windows (GraphPad Software, Boston, MA, USA, www.graphpad.com [accessed on 25 August 2025]).

3. Results

Of the 87 sites that participated in the core PANORAMA phase 3 trial, 14 sites (16%) participated in VOYAGE and enrolled patients in the prospective VOYAGE extension study (full list of participating sites in Table S1). Of the 111 patients completing PANORAMA at these sites who were potentially eligible for enrollment, 41 patients (37%) enrolled into VOYAGE from June 2021 to July 2022, constituting 13% of the total 320 patients who completed PANORAMA (Figure 1). The demographics of the VOYAGE patients were consistent with those of the overall PANORAMA population, with all 3 original randomized arms being approximately equally represented within VOYAGE (Table 1). The total mean (SD) time of follow-up from initial enrollment in PANORAMA to the 1-year endpoint of VOYAGE was 5.7 years (0.4), or approximately 5 years and 8 months.

Table 1. Demographic and ocular characteristics of patients in VOYAGE and PANORAMA.

	Prospective VOYAGE Enrollment *		**PANORAMA Enrollment †**			**Enrollment Baseline Totals**	
	Group 1 (*n* = 35)	**Group 2 § (*n* = 6)**	**Sham (*n* = 11)**	**Q16W ¥ (*n* = 12)**	**Q8W (*n* = 18)**	**VOYAGE ‡ (*n* = 41)**	**PANORAMA (*n* = 402)**
Age, mean (SD), yrs	59.4 (12.0)	59.0 (6.3)	63.4 (8.9)	58.1 (14.7)	57.6 (10.0)	59.3 (11.3)	55.7 (10.5)
Male, *n* (%)	19 (54)	2 (33)	5 (46)	6 (50)	10 (56)	21 (51)	225 (56)
Race, *n* (%)							
White	30 (86)	6 (100)	10 (91)	12 (100)	14 (78)	36 (88)	310 (77)
Black	4 (11)	0	1 (9)	0	3 (17)	4 (10)	41 (10)
Asian	1 (3)	0	0	0	1 (6)	1 (2)	23 (6)
Other	0	0	0	0	0	0	28 (7)
Hispanic or Latino, *n* (%)	19 (54)	1 (17)	7 (64)	6 (50)	7 (39)	20 (49)	Not reported
HbA1c, mean (SD), %	8.6 (2.2)	10.2 (3.0)	8.9 (2.7)	8.4 (2.3)	9.2 (2.3)	8.9 (2.4)	8.5 (1.6)
ETDRS letters, mean (SD)	79.3 (8.7)	65.5 (32.2)	82.2 (6.8)	73.8 (23.8)	76.7 (9.9)	77.3 (14.8)	82.4 (6.0)
Snellen equivalent	20/25	20/50	20/25	20/32	20/32	20/32	20/25
CST, mean (SD), μm	291.9 (67.7)	308.0 (75.4)	279.9 (50.4)	294.9 (42.8)	301.9 (88.4)	293.9 (67.9)	247.4 (34.8)
DRSS level, *n* (%)							
≤43	25 (71)	0	6 (55)	6 (50)	13 (72)	25 (61)	0
47	1 (3)	0	0	1 (8)	0	1 (2)	302 (75)
53	1 (3)	0	0	1 (8)	0	1 (2)	100 (25)
≥60	8 (23)	5 (100)	5 (45)	3 (25)	5 (28)	13 (32)	0

CST = central subfield thickness; DRSS = diabetic retinopathy severity scale; ETDRS = Early Treatment Diabetic Retinopathy Study; HbA1c = glycosylated hemoglobin; SD = standard deviation. * Patients assigned to Group 1 (no history of panretinal photocoagulation) and Group 2 (history of panretinal photocoagulation) during VOYAGE. † Patients initially randomized to the sham, every-16-week (Q16W), and every-8-week (Q8W) arms during PANORAMA. ‡ CST *n* = 40 due to 1 case of vitreous hemorrhage at VOYAGE baseline; DRSS *n* = 40 due to 1 case of DRSS level not measured at VOYAGE baseline. § DRSS *n* = 5 due to 1 case of DRSS level not measured at VOYAGE baseline. ¥ DRSS *n* = 11 due to 1 case of DRSS level not measured at VOYAGE baseline.

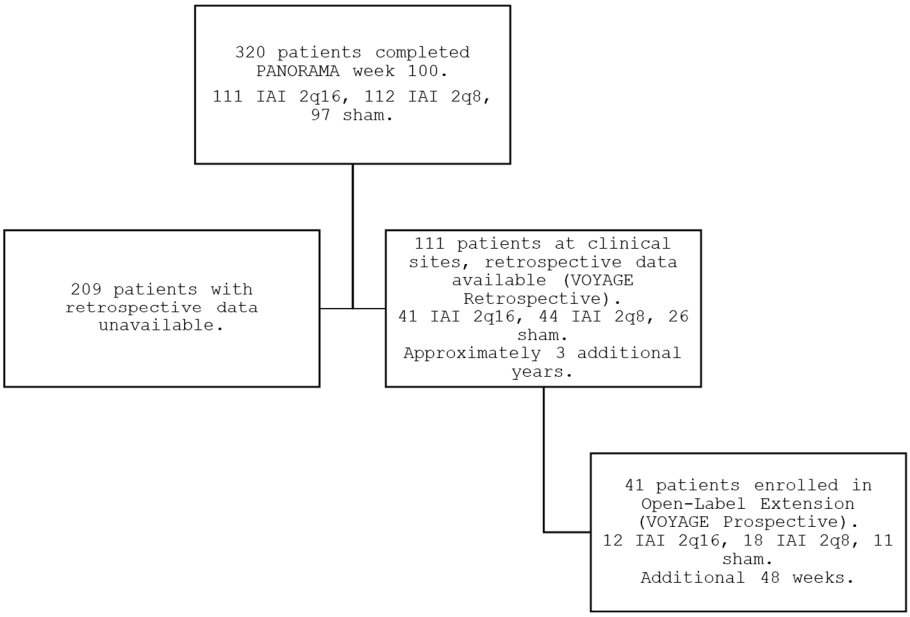


Figure 1. PANORAMA, interim period, and VOYAGE study flow.

3.1. Clinical Course During Interim Period

Among the 41 patients enrolled, the mean length of the interim period from PANORAMA exit to VOYAGE enrollment was 33.7 months (SD 4.8, range 23.9–44.2). During this period, 24 patients (59%, 24/41) received no anti-VEGF injections, while the other 17 patients (41%, 17/41) received a mean of 2.7 anti-VEGF injections per year. Among all patients, a mean of 1.1 injections per year (SD 1.9) were given during the interim period (Figure 2). Patients who had been in the combined aflibercept arms within PANORAMA (n = 30) experienced significant decreases in annualized mean anti-VEGF injections from 5.6 injections per year (SD 1.3) during PANORAMA to 1.0 injections per year (SD 1.6) in the interim ($p < 0.001$), whereas sham patients within PANORAMA (n = 11) did not have significantly different anti-VEGF injection rates during PANORAMA due to the institution of rescue therapy (2.7 injections per year [SD 3.9]) compared to the interim period (1.4 injections per year [SD 2.6]) (p = 0.36).

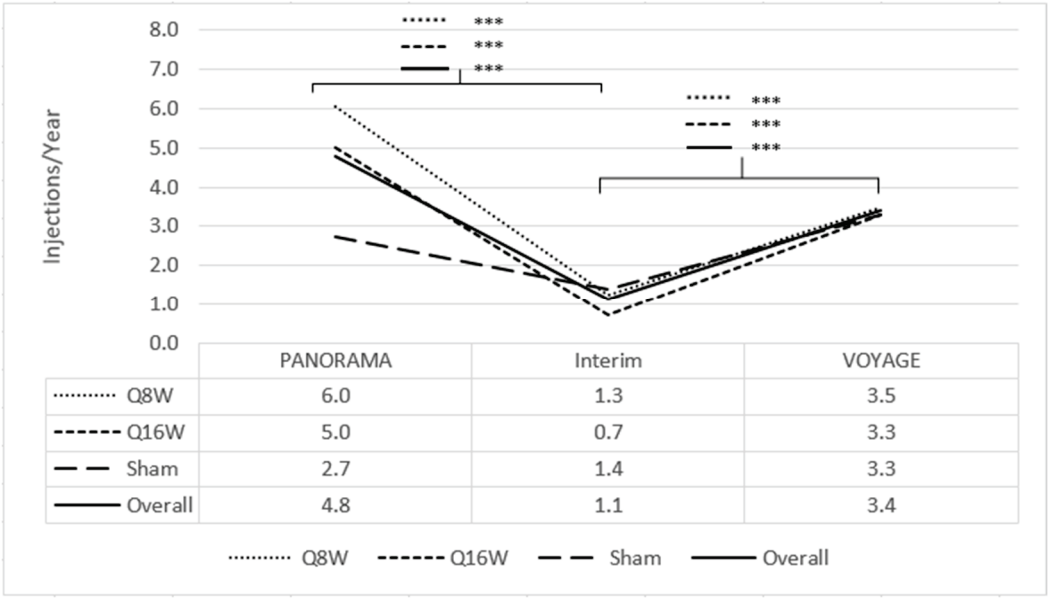


Figure 2. Mean injection frequency from PANORAMA enrollment to VOYAGE 1-year, annualized. The line corresponding to *** signifies statistical significance of <0.001 on a two-tailed t-test.

Three patients (7%, 3/41) received PRP during the interim period. Nine patients (22%, 9/41) received focal macular laser during the interim period. One patient (2%, 1/41) underwent pars plana vitrectomy (PPV) during the interim period for vitreous hemorrhage.

At PANORAMA exit, 73% (8/11) and 89% (24/27) of the sham and combined aflibercept arms had DRSS level $\leq$ 43, respectively. During the interim period, 41% (15/37) of patients worsened, of which 5 patients worsened 1 step and 10 patients worsened $\geq$ 2 steps; 13 patients (35%, 13/37) had stable DRSS levels; and 9 (24%, 9/37) improved by 1 or 2 steps at VOYAGE enrollment compared to PANORAMA exit (Figure 3). Notably, the proportion of patients with PDR (DRSS level $\geq$ 60) increased from 5% (2/38) to 33% (13/40), while the proportion of patients with DRSS level $\leq$ 43 decreased from 84% (32/38) at the beginning of the interim period to 63% (25/40) at the end. Additionally, mean HbA1c remained stable during the interim period (8.9% [SD 1.8] at PANORAMA exit and 8.9% [SD 2.4] at VOYAGE enrollment). Seven patients (18%, 7/39) had center-involving DME by the end of the interim period.

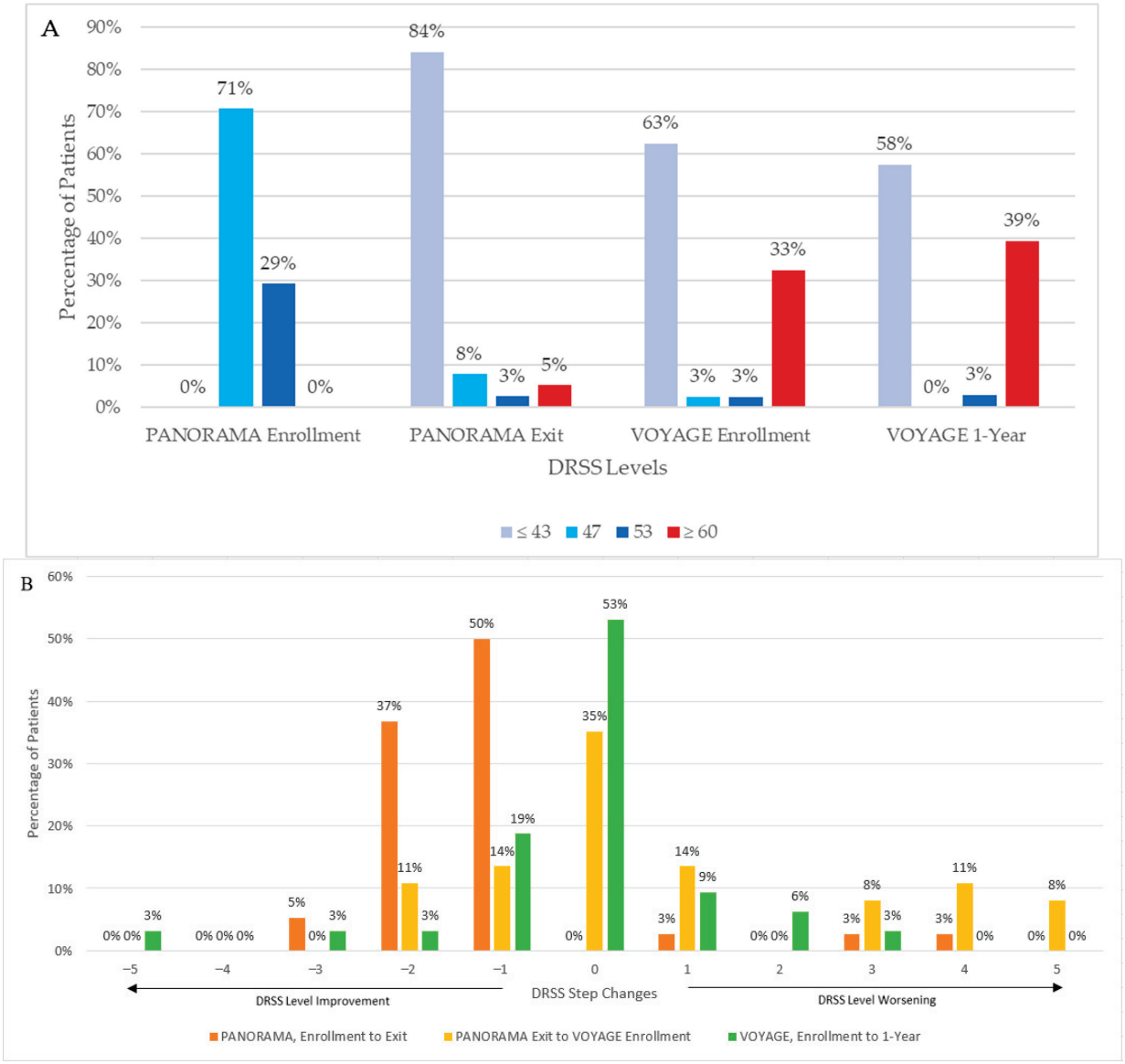
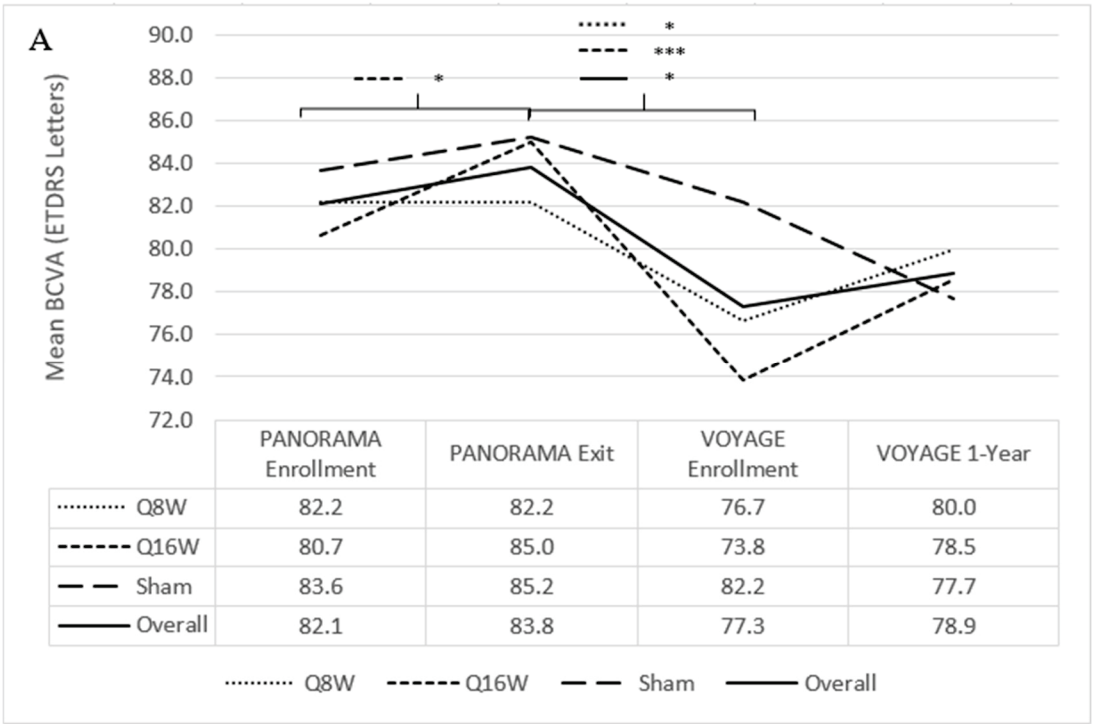


Figure 3. Diabetic Retinopathy Severity Scale (DRSS) levels from PANORAMA enrollment to VOYAGE 1-year. (**A**) Absolute DRSS levels. (**B**) Step changes in DRSS levels; positive values represent DRSS step worsening to a greater severity, and negative values represent DRSS step improvements to a lower severity.

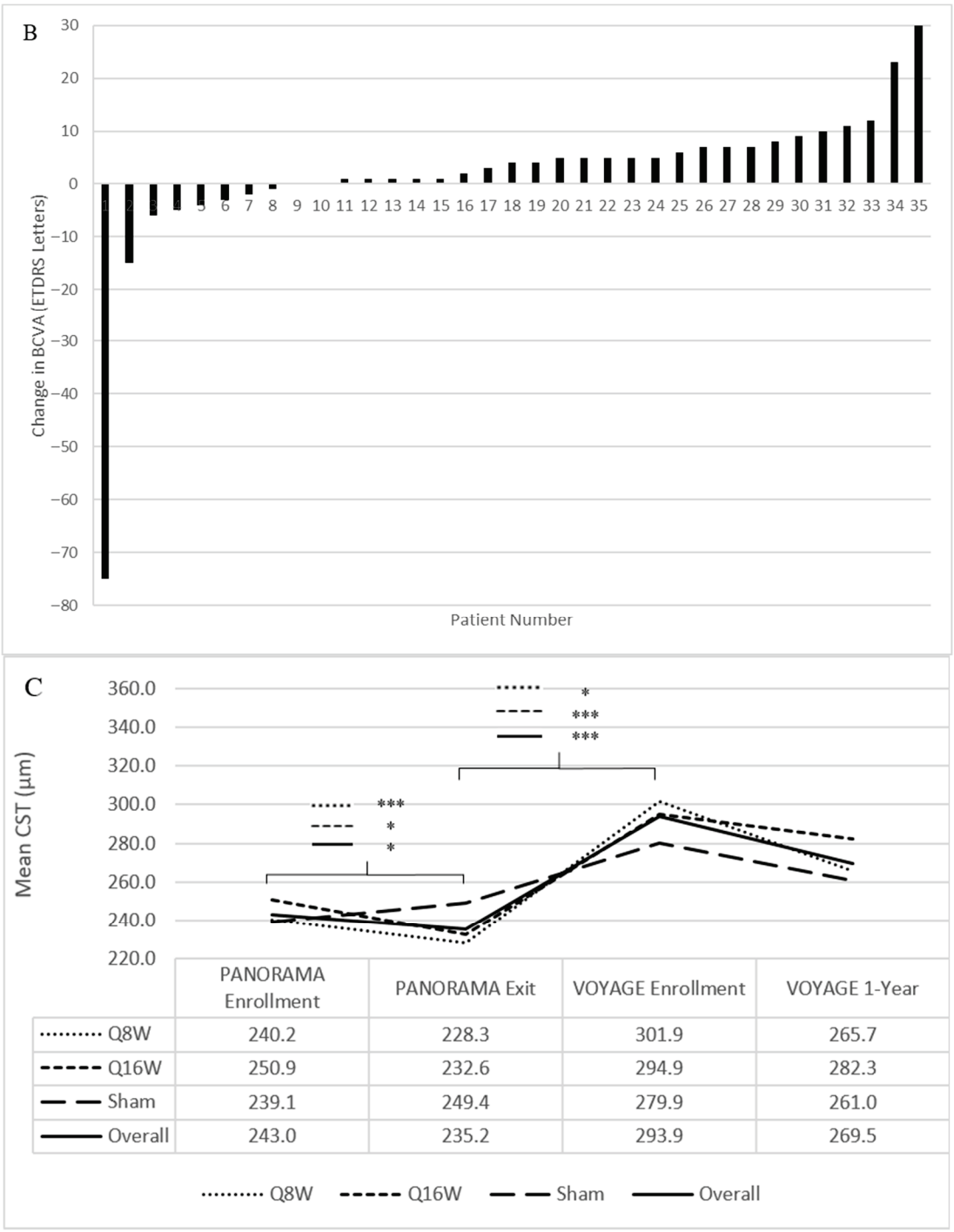
Sub-analysis of the 22 patients (59%, 22/37) who experienced stable or improved DRSS levels during the interim period revealed a roughly equal distribution of patients assigned to PANORAMA Q16W, Q8W, and sham arms (5:10:7); 15 of the 22 patients (68%) had a PANORAMA baseline DRSS level of 47, and the other 7 patients (32%) had a baseline DRSS level of 53. Of the patients with stable or improved DRSS levels during the interim period, 20 patients (91%, 20/22) were in Group 1, and the other 2 patients (9%, 2/22) were in Group 2. This is a similar distribution as that of the 15 patients who experienced worsening DRSS levels during the interim period, with 87% (13/15) in Group 1 and 13% (2/15) in Group 2. There was no meaningful difference in the proportion of patients who improved, remained stable, or worsened during VOYAGE compared with their previous mean anti-VEGF injection frequency during PANORAMA (one-way ANOVA, $p > 0.05$). There were slight differences during the interim period (one-way ANOVA with multiple comparisons, $p = 0.04$): 2.1 greater annual injections for patients who improved compared to remained stable ($p = 0.04$), and 1.8 greater annual injections for patients who improved compared to worsened ($p > 0.05$), while patients who remained stable compared to worsened received approximately the same number of injections ($p > 0.05$).

At PANORAMA exit, mean BCVA was not significantly different between the combined aflibercept and sham arms. Mean BCVA decreased during the interim period from 83.8 letters (SD 6.1) to 77.3 letters (SD 14.8) ($p = 0.009$), representing a mean loss of 6.5 letters (Figure 4A). BCVA losses during the interim period were most notable among patients who had been in the combined aflibercept arms, with combined mean BCVA decreasing by 7.8 mean letters, from 83.3 letters (SD 5.9) at PANORAMA exit to 75.5 letters (SD 16.6) at VOYAGE enrollment ($p = 0.02$). BCVA losses were less among patients previously enrolled in the sham arm (85.2 letters [SD 6.6] to 82.2 letters [SD 6.8]). Patients from all of the PANORAMA arms combined who remained in the NPDR stage ($n = 26$, DRSS level ≤ 53) throughout the interim period also experienced a mean 4.3 letter loss from 85.5 letters (SD 4.2) at PANORAMA exit to 81.2 letters (7.0) at VOYAGE enrollment (paired, $p < 0.001$).



	PANORAMA Enrollment	PANORAMA Exit	VOYAGE Enrollment	VOYAGE 1-Year
Q8W	82.2	82.2	76.7	80.0
Q16W	80.7	85.0	73.8	78.5
Sham	83.6	85.2	82.2	77.7
Overall	82.1	83.8	77.3	78.9

Figure 4. *Cont.*



	PANORAMA Enrollment	PANORAMA Exit	VOYAGE Enrollment	VOYAGE 1-Year
Q8W	240.2	228.3	301.9	265.7
Q16W	250.9	232.6	294.9	282.3
Sham	239.1	249.4	279.9	261.0
Overall	243.0	235.2	293.9	269.5

Figure 4. Mean best-corrected visual acuity (BCVA) and central subfield thickness (CST) from PANORAMA enrollment to VOYAGE 1-year. (**A**) Mean BCVA from PANORAMA enrollment to VOYAGE 1-year. (**B**) Change in BCVA from VOYAGE baseline to VOYAGE 1-year for each patient. Notably, patients 1, 2, 29, and 35 had a traumatic cataract resulting in 0 letters read at VOYAGE 1-year, endophthalmitis resulting in a loss of 15 letters by VOYAGE 1-year, cataract extraction resulting in an improvement of 8 letters at VOYAGE 1-year, and vitreous hemorrhage resulting in 0 letters read at VOYAGE baseline, respectively. (**C**) Mean CST from PANORAMA enrollment to VOYAGE 1-year. ETDRS = Early Treatment Diabetic Retinopathy Study. Line-corresponding * signifies statistical significance of $p < 0.05$, and *** signifies statistical significance of $p < 0.001$ on a two-tailed *t*-test.

At PANORAMA exit, mean CST was not significantly different between the combined aflibercept and sham arms. Mean CST during the interim period increased from 235.2 µm

(SD 39.1) to 293.9 μm (SD 67.9) ($p < 0.001$) (Figure 4C). Changes in CST during the interim period were most notable among patients who had been in the combined aflibercept arms, with combined mean CST increasing by 69.2 μm, from 230.0 μm (SD 32.6) to 299.2 μm (SD 73.5) ($p < 0.001$); patients previously enrolled in the sham arm experienced a smaller increase in mean CST of 30.5 μm, from 249.4 μm (SD 52.4) to 279.9 μm (SD 50.4).

3.2. Clinical Course During VOYAGE

Thirty-five patients (85%, 35/41) completed the VOYAGE 1-year primary endpoint; 2 patients died (due to myocardial infarction (MI) and unknown cause), 2 were lost to follow-up, and 2 withdrew consent at week 16 and 48 due to hospitalization following a cerebrovascular accident (CVA) and patient preference, respectively. Of the 164 possible visits among all patients through the first year, 14 visits (9%) were missed.

Through 1 year of VOYAGE, 7 patients (17%, 7/41) received no 2 mg intravitreal aflibercept injections and the remaining 34 (83%, 34/41) received a mean of 3.5 injections (range 1–9 injections); among the entire population, mean injection frequency increased from 1.1 injections per year (SD 1.9) during the interim period to 3.4 injections per year (SD 2.6) during VOYAGE ($p < 0.001$) (Figure 2). Of the patients who had been in the combined aflibercept arms, 2 mg intravitreal aflibercept injection frequency increased from 1.0 injection per year (SD 1.6) during the interim to 3.4 injections per year (SD 2.7) during VOYAGE ($p < 0.001$); patients who had been in the sham arm did not experience a significant change in 2 mg intravitreal aflibercept injection frequency from the interim period (1.4 injections per year [SD 2.6]) to VOYAGE (3.3 injections per year [SD 2.2]) ($p = 0.09$).

There were no meaningful differences in the annualized mean injection frequencies between Groups 1 (3.3 injections per year [SD 2.4]) and 2 (3.7 injections per year [SD 3.5]), or among patients previously assigned to the sham, Q16W, and Q8W arms (mean of 3.3 injections per year [SD 2.2], 3.3 injections per year [SD 3.3], and 3.5 injections per year [SD 2.3], respectively) during VOYAGE. One patient (2%, 1/41) received PRP during year 1 of VOYAGE, and 1 patient (2%, 1/41) received PPV for vitreous hemorrhage.

Mean HbA1c decreased from 8.9% (SD 2.4) at VOYAGE enrollment to 8.1% (SD 1.9) at VOYAGE 1 year. Eleven patients (27%, 11/41) were on glucagon-like peptide-1 receptor agonists during VOYAGE, with no apparent meaningful differences in baseline or 1-year outcomes between these patients and the rest of the VOYAGE cohort.

3.2.1. Diabetic Retinopathy Severity Scale Levels

Overall, DRSS levels appeared relatively stable through 1 year of VOYAGE; 53% (17/32) did not change DRSS level, while 28% (9/32) improved and 19% (6/32) worsened (Figure 3). The proportion of patients with DRSS level ≤ 43 decreased from 63% (25/40) to 58% (19/33), and the proportion of patients with DRSS level ≥ 60 increased from 33% (13/40) to 39% (13/33) from VOYAGE enrollment to year 1.

Of the patients who had PDR (DRSS level ≥ 60; 33%, 13/40) at VOYAGE enrollment, 42% (5/12) improved, 42% (5/12) remained stable, and 17% (2/12) worsened through 1 year. Of the patients who had NPDR (68%, 27/40) at VOYAGE enrollment, 20% (4/20) improved, 60% (12/20) remained stable, and 20% (4/20) worsened through 1 year. One of the patients (5%, 1/20) with NPDR progressed to PDR at 1 year. Four (20%, 4/20) of the patients with NPDR improved by at least 1 step by VOYAGE 1-year. Only 1 patient (3%, 1/32) developed new center-involving DME through VOYAGE 1-year.

The 6 patients (19%, 6/32) with worse DRSS levels through 1 year had mild to moderate NPDR (67%, 4/6) or inactive PDR (33%, 2/6) at VOYAGE baseline. In comparison, 26 patients (81%, 26/32) experienced DRSS level stability or improvement throughout 1 year of VOYAGE, with 23 patients (72%, 23/32) in Group 1 and 3 (9%, 3/32) in Group 2.

There were no meaningful differences ($p > 0.05$) in mean HbA1c at VOYAGE initiation or 1 year, mean 2 mg intravitreal aflibercept injections received in VOYAGE at 1 year, or cumulative mean 2 mg intravitreal aflibercept injections per year since PANORAMA initiation between patients who improved or worsened in DRSS levels at VOYAGE 1-year.

3.2.2. Best-Corrected Visual Acuity and Central Subfield Thickness

Mean BCVA remained stable from VOYAGE baseline (77.3 letters [SD 14.8]) to 1 year (78.9 letters [SD 17.7]) (Figure 4A). In total, 27 patients (77%, 27/35) had stable or improved BCVA through 1 year. At VOYAGE baseline, 1 patient (2%, 1/41) had a vitreous hemorrhage, resulting in 0 letters read (Figure 4B). One patient (3%, 1/35) received cataract surgery while enrolled in VOYAGE and subsequently experienced an 8-letter improvement at 1 year. At VOYAGE 1-year, 1 patient (3%, 1/35) had a traumatic cataract resulting in 0 letters read. Five patients (14%, 5/35) gained at least 10 letters from VOYAGE baseline through 1 year, and 1 patient (3%, 1/35) lost 15 letters following endophthalmitis.

At VOYAGE 1-year, mean CST decreased from 293.9 μm (SD 67.9) to 269.5 μm (SD 34.5) ($p = 0.06$) (Figure 4C). Twenty-one patients (64%, 21/33) had improved CST through 1 year. There was no significant difference in CST decrease among patients who had PDR (46.2 μm [SD 105.3]) versus NPDR at VOYAGE baseline (10.6 μm [SD 40.0]), or between Group 1 (22.3 μm [SD 71.3]) and Group 2 (32.3 μm [SD 84.0]) patients. Patients previously assigned to sham, Q16W, and Q8W during PANORAMA had mean decreases in CST of 13.8 μm (SD 36.1), 13.7 μm (SD 62.4), and 36.8 μm (SD 93.6), respectively, which also did not significantly differ from one another.

3.2.3. Adverse Events

Through 1 year of VOYAGE, treatment-emergent AEs occurred in 28 patients (68%, 28/41), none of which were determined to be related to aflibercept. There was 1 case of traumatic cataract related to the injection procedure at week 48 out of 119 total injections (incidence of 0.8%), which included 72 injections in phakic eyes (incidence of 1% of phakic injections), during the prospective component of VOYAGE. Inflammatory events were reported in 2 patients (5%, 2/41), with 1 case of concurrent anterior chamber cells and vitreous haze, which were reportedly related to keratoconjunctivitis sicca and active PDR, respectively, and did not require treatment, and 1 case of endophthalmitis (incidence of 1/119 injections, or 0.8%) reported as related to the injection procedure. Reported serious AEs included 1 vascular death from a fatal MI (2%, 1/41), 1 death of unknown cause (2%, 1/41), and 1 nonfatal CVA (2%, 1/41).

4. Discussion

Results from the prospective VOYAGE study, which enrolled a subset of patients who completed the PANORAMA phase 3 trial, indicate that during an approximate 3-year period of routine clinical care, DRSS level, CST, and BCVA all appeared to numerically worsen, particularly among patients who had been randomized to receive 2 mg intravitreal aflibercept injection during PANORAMA. Concurrently, there was a decrease in intravitreal anti-VEGF injections administered during the interim period. Then, following initiation of aflibercept therapy for pre-defined DR activity thresholds during VOYAGE, overall mean BCVA and CST increased by 1.8 letters and decreased by 24.4 μm, respectively. Relevant to this reversal, patients received intravitreal anti-VEGF injections more than 4 times as frequently during PANORAMA (4.8 injections per year) and almost 3 times as frequently during VOYAGE (3.4 injections per year) compared to the interim period (1.1 injections per year).

From PANORAMA baseline to the VOYAGE 1-year timepoint, DRSS levels worsened for many patients, with 39% (13/33) of patients developing PDR. This DRSS level worsening occurred predominantly during the interim period, when less frequent anti-VEGF treatment was utilized, and DRSS levels predominantly stabilized with more frequent treatment during VOYAGE. More frequent anti-VEGF dosing may have been needed to achieve maximal DRSS level improvement. Furthermore, the ability to improve DRSS levels may be at least partially dependent on initial disease severity; specifically, in some datasets, DRSS level improvements have been less robust among patients with PDR compared to those with NPDR [12,13]. For example, in CLARITY, 78% of patients treated with a mean of 4.4 aflibercept injections through 1 year did not improve to NPDR [12]; VOYAGE seemed to produce similar results, with 11 of the patients (92%, 11/12) with PDR not returning to NPDR following 1 year of aflibercept treatment. Additionally, there were no meaningful differences in mean HbA1c at VOYAGE baseline or 1 year between patients who experienced improved or worsened DRSS levels at VOYAGE 1-year. Larger studies with longer follow-up time are needed to better identify patient-level factors that may influence who benefits most from sustained aflibercept therapy.

Considering BCVA and CST longitudinally, although both appeared relatively stable between PANORAMA baseline and the VOYAGE 1-year timepoint, there were periods of substantial fluctuation throughout the 5.7 years in between. These changes appear to have been driven by the meaningfully different approaches to clinical care utilized during these 3 time periods. Specifically, during the interim period when patients received routine care outside of a rigid prospective protocol, VA and CST temporarily worsened by over 6 ETDRS letters and 58 μm, respectively. Of note, fluctuations in specific DME biomarkers have been reported to be associated with worse visual outcomes. For example, greater CST fluctuations have been associated with worse VA outcomes at 1 and 2 years among DME patients in retrospective analyses [14,15]. While the visit-to-visit granularity of CST fluctuations is beyond the scope of the current work, overall data suggests that in order to maximize clinical outcomes, it may be worthwhile to consider a personalized approach with more consistent intervention in order to minimize disease fluctuations.

During the interim period, when less structured follow-ups and less consistent anti-VEGF dosing were utilized, clinical characteristics appeared to worsen. According to the 2024 American Society of Retina Specialists Preferences and Trends Survey, inconsistent or insufficient office visits were found to be the factor that has the greatest negative impact on all patient outcomes in the United States [16]. However, providing consistent anti-VEGF therapy to patients in a real-world setting is challenging due to a multitude of factors [17]. Additionally, intravitreal injections carry risk as evidenced by the cases of infectious endophthalmitis and traumatic cataract observed in the current series. In order to address these meaningful hurdles to optimal outcomes and care delivery, multiple therapeutic approaches currently under investigation seek to provide consistent, longitudinal ocular-specific treatment for DR and other chronic, exudative retinal diseases. These include anti-VEGF port delivery systems [18], extended durability agents such as tyrosine kinase inhibitors [19,20], and anti-VEGF gene therapies such as RGX-314 and ADVM-022, both of which have demonstrated durable intraocular anti-VEGF protein production and exudative disease control following subretinal and intravitreal delivery, respectively [21–23]. For any potential more durable therapy, the risks and benefits will need to be considered in the clinical context of each patient.

Limitations of the current work include that of the 320 patients across 5 countries who completed PANORAMA, only 41 patients (13% of PANORAMA completers) enrolled in VOYAGE, and all patients were from United States sites, which may limit the generalizability of the results. The underlying reasons for this are likely multifactorial,

including study-site operational limitations, patients lost to follow-up, and the additional significant time commitment after having already participated in PANORAMA. While this low VOYAGE enrollment number raises concerns about selection bias and whether this cohort accurately reflects the entire PANORAMA patient population, no meaningful differences were observed in PANORAMA baseline characteristics between the subset that enrolled in VOYAGE and the full PANORAMA cohort. Additionally, although there were no apparent outcome differences between Group 1 and Group 2 patients, the cohort of patients in Group 2 was small (n = 6), which limited the overall ability to meaningfully compare these groups.

Additionally, a key challenge when assessing aflibercept efficacy and durability are the specific criteria used to determine when and how often to treat. VOYAGE treatment criteria were designed to allow physicians to use clinical judgment as to the necessity of retreatment in cases of DME. While this better reflects a real-world approach, it also generates greater variability in treatment decisions and therefore may have influenced variability in outcomes. Future studies using standardized treatment criteria may reduce this variability and improve consistency among outcomes. Another interesting result from this real-world approach was the observation that, on an individual-patient basis, clinician-determined DRSS levels and reading center-determined DRSS levels sometimes disagreed; whereas investigators' scores were distributed more evenly across all NPDR severity levels, the reading center leaned heavily toward mild NPDR scores. Across all patients enrolled, comparing investigator to reading center scores did not change the results, particularly regarding the number of PDR patients. This discrepancy may be due to various factors, such as access to information or time constraints in the clinic setting.

Another key limitation is that because the treatment regimens during the interim period varied considerably, it is challenging to compare how much of the observed anatomic and functional decline was attributable to undertreatment versus disease progression. Additionally, all analyses utilized observed data, so meaningful outliers may have impacted mean values. For example, true mean BCVA improvement may have been underestimated due to the inclusion of 1 case (3%, 1/35) of 0 letters read at 1 year due to traumatic cataract, resulting in an apparent 75 letter loss from VOYAGE baseline through 1 year. Outliers were described when results were meaningfully impacted.

In summary, the observed changes in ocular characteristics across the mean 5.7 years from PANORAMA enrollment through the VOYAGE 1-year endpoint appeared to correlate with anti-VEGF dosing frequency. Patients who received less frequent 2 mg intravitreal aflibercept injections after PANORAMA exit experienced a trend toward worsening of DRSS level, BCVA, and CST at VOYAGE enrollment, trends which appeared to stabilize or improve after re-initiating regular 2 mg intravitreal aflibercept injections during VOYAGE. More data on long-term outcomes are needed from larger patient populations with NPDR to assess the sustainability of these treatment effects and to adequately inform clinical management of this common, chronic condition. Finally, new, safe therapeutics which meaningfully reduce treatment burden may be valuable for personalized, long-term DR management.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jpm15110555/s1, Table S1. Participating sites in PANORAMA and VOYAGE.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Advarra Institutional Review Board (Columbia, MD, United States) on 31 March 2021 (IRB approval Pro00044668). The study's clinical trial registration number is identifier NCT04708145 registered with ClinicalTrials.gov on 12 January 2021.

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Data Availability Statement: The data collected in the course of this study is not publicly available due to it containing information that could compromise the privacy of research patients. Further inquiries can be directed to the corresponding author.

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Abbreviations

The following abbreviations are used in this manuscript:

AE	Adverse event
ANOVA	Analysis of variance
BCVA	Best-corrected visual acuity
CST	Central subfield thickness
CVA	Cerebrovascular accident
DME	Diabetic macular edema
DR	Diabetic retinopathy
DRSS	Diabetic retinopathy severity scale
ETDRS	Early treatment diabetic retinopathy study
HbA1c	Glycosylated hemoglobin
MI	Myocardial infarction
NPDR	Non-proliferative diabetic retinopathy
OCT	Optical coherence tomography
PDR	Proliferative diabetic retinopathy

PPV	Pars plana vitrectomy
PRN	Pro re nata (as needed)
PRP	Panretinal photocoagulation
Q16W	Every-16-week aflibercept following 3 initial monthly doses and one 8-week interval through 2 years
Q8W	Every-8-week aflibercept following 5 initial monthly doses with pro re nata dosing in year 2 if diabetic retinopathy severity scale level was worse than 35
SD	Standard deviation
VA	Visual acuity
VEGF	Vascular endothelial growth factor

References

1. Lundeen, E.A.; Burke-Conte, Z.; Rein, D.B.; Wittenborn, J.S.; Saaddine, J.; Lee, A.Y.; Flaxman, A.D. Prevalence of Diabetic Retinopathy in the US in 2021. *JAMA Ophthalmol.* **2023**, *141*, 747–754. [CrossRef]
2. Wang, W.; Lo, A.C.Y. Diabetic Retinopathy: Pathophysiology and Treatments. *Int. J. Mol. Sci.* **2018**, *19*, 1816. [CrossRef]
3. Early Treatment Diabetic Retinopathy Study Research Group. Fundus Photographic Risk Factors for Progression of Diabetic Retinopathy: ETDRS Report Number 12. *Ophthalmology* **1991**, *98*, 823–833. [CrossRef]
4. Klein, R.; Klein, B.E.K.; Moss, S.E. How Many Steps of Progression of Diabetic Retinopathy Are Meaningful?The Wisconsin Epidemiologic Study of Diabetic Retinopathy. *Arch. Ophthalmol.* **2001**, *119*, 547–553. [CrossRef] [PubMed]
5. Maturi, R.K.; Glassman, A.R.; Josic, K.; Baker, C.W.; Gerstenblith, A.T.; Jampol, L.M.; Meleth, A.; Martin, D.F.; Melia, M.; Punjabi, O.S.; et al. Four-Year Visual Outcomes in the Protocol W Randomized Trial of Intravitreous Aflibercept for Prevention of Vision-Threatening Complications of Diabetic Retinopathy. *JAMA* **2023**, *329*, 376–385. [CrossRef] [PubMed]
6. Brown, D.M.; Wykoff, C.C.; Boyer, D.; Heier, J.S.; Clark, W.L.; Emanuelli, A.; Higgins, P.M.; Singer, M.; Weinreich, D.M.; Yancopoulos, G.D.; et al. Evaluation of Intravitreal Aflibercept for the Treatment of Severe Nonproliferative Diabetic Retinopathy: Results From the PANORAMA Randomized Clinical Trial. *JAMA Ophthalmol.* **2021**, *139*, 946–955. [CrossRef] [PubMed]
7. Korobelnik, J.F.; Do, D.V.; Schmidt-Erfurth, U.; Boyer, D.S.; Holz, F.G.; Heier, J.S.; Midena, E.; Kaiser, P.K.; Terasaki, H.; Marcus, D.M.; et al. Intravitreal Aflibercept for Diabetic Macular Edema. *Ophthalmology* **2014**, *121*, 2247–2254. [CrossRef]
8. Gross, J.G.; Glassman, A.R.; Liu, D.; Sun, J.K.; Antoszyk, A.N.; Baker, C.W.; Bressler, N.M.; Elman, M.J.; Ferris, F.L.; Gardner, T.W.; et al. Five-Year Outcomes of Panretinal Photocoagulation vs Intravitreous Ranibizumab for Proliferative Diabetic Retinopathy: A Randomized Clinical Trial. *JAMA Ophthalmol.* **2018**, *136*, 1138–1148. [CrossRef]
9. Boyer, D.S.; Nguyen, Q.D.; Brown, D.M.; Basu, K.; Ehrlich, J.S. Outcomes with As-Needed Ranibizumab after Initial Monthly Therapy: Long-Term Outcomes of the Phase III RIDE and RISE Trials. *Ophthalmology* **2015**, *122*, 2504–2513.e1. [CrossRef]
10. Wykoff, C.C.; Ou, W.C.; Khurana, R.N.; Brown, D.M.; Lloyd Clark, W.; Boyer, D.S. Long-term outcomes with as-needed aflibercept in diabetic macular oedema: 2-year outcomes of the ENDURANCE extension study. *Br. J. Ophthalmol.* **2018**, *102*, 631–636. [CrossRef]
11. World Medical Association. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. *JAMA* **2013**, *310*, 2191–2194. [CrossRef]
12. Sivaprasad, S.; Prevost, A.T.; Vasconcelos, J.C.; Riddell, A.; Murphy, C.; Kelly, J.; Bainbridge, J.; Tudor-Edwards, R.; Hopkins, D.; Hykin, P.; et al. Clinical efficacy of intravitreal aflibercept versus panretinal photocoagulation for best corrected visual acuity in patients with proliferative diabetic retinopathy at 52 weeks (CLARITY): A multicentre, single-blinded, randomised, controlled, phase 2b, non-inferiority trial. *Lancet* **2017**, *389*, 2193–2203. [CrossRef] [PubMed]
13. Wykoff, C.C.; Eichenbaum, D.A.; Roth, D.B.; Hill, L.; Fung, A.E.; Haskova, Z. Ranibizumab Induces Regression of Diabetic Retinopathy in Most Patients at High Risk of Progression to Proliferative Diabetic Retinopathy. *Ophthalmol. Retina.* **2018**, *2*, 997–1009. [CrossRef] [PubMed]
14. Starr, M.R.; Salabati, M.; Mahmoudzadeh, R.; Patel, L.G.; Ammar, M.J.; Hsu, J.; Garg, S.; Ho, A.C.; Kuriyan, A.E. Fluctuations in Central Subfield Thickness Associated With Worse Visual Outcomes in Patients with Diabetic Macular Edema in Clinical Trial Setting. *Am. J. Ophthalmol.* **2021**, *232*, 90–97. [CrossRef] [PubMed]
15. Wang, V.Y.; Kuo, B.L.; Chen, A.X.; Wang, K.; Greenlee, T.E.; Conti, T.F.; Singh, R.P. Fluctuations in macular thickness in patients with diabetic macular oedema treated with anti-vascular endothelial growth factor agents. *Eye* **2022**, *36*, 1461–1467. [CrossRef]
16. Hahn, P.; Garg, S.J. (Eds.) *2024 Global Trends in Retina Survey*; American Society of Retina Specialists: Chicago, IL, USA, 2024.
17. Suresh, R.; Yu, H.J.; Thoveson, A.; Swisher, J.; Apolinario, M.; Zhou, B.; Shah, A.R.; Fish, R.H.; Wykoff, C.C. Loss to Follow-Up Among Patients With Proliferative Diabetic Retinopathy in Clinical Practice. *Am. J. Ophthalmol.* **2020**, *215*, 66–71. [CrossRef]

18. Emanuelli, A.; Holekamp, N.M.; Latkany, P.; Hu, A.Y.; Suñer, I.J.; Wykoff, C.C.; Brittain, C.; Howard, D.; Quezada-Ruiz, C.; Santhanakrishnan, A.; et al. Port delivery system with ranibizumab in the treatment of diabetic retinopathy without center-involved diabetic macular edema: Primary analysis results of the phase 3 Pavilion trial. *Invest. Ophthalmol. Vis. Sci.* **2023**, *64*, 3754.
19. Study to Evaluate the Safety, Tolerability, and Efficacy of OTX-TKI in Subjects With Moderately Severe to Severe Non-Proliferative Diabetic Retinopathy. ClinicalTrials.gov Identifier: NCT05695417. Updated 8 December 2023. Available online: https://clinicaltrials.gov/study/NCT05695417 (accessed on 25 July 2024).
20. Eichenbaum, D.A.; Hershberger, V.; Patel, S.S.; Abbey, A.M.; Bridges, W.Z.; Khanani, A.M.; Ruggiero, S.; Roy, M.; Duker, J.S.; Paggiarino, D.A.; et al. The DAVIO 2 trial: A phase 2, randomized, double-masked, controlled multicenter study of EYP-1901 vs. aflibercept in previously treated wet age-related macular degeneration. *Investig. Ophthalmol. Vis. Sci.* **2024**, *65*, 4401.
21. Campochiaro, P.A.; Avery, R.; Brown, D.M.; Heier, J.S.; Ho, A.C.; Huddleston, S.M.; Jaffe, G.J.; Khanani, A.M.; Pakola, S.; Pieramici, D.J.; et al. Gene therapy for neovascular age-related macular degeneration by subretinal delivery of RGX-314: A phase 1/2a dose-escalation study. *Lancet* **2024**, *403*, 1563–1573. [CrossRef]
22. Gelfman, C.M.; Grishanin, R.; Bender, K.O.; Nguyen, A.; Greengard, J.; Sharma, P.; Nieves, J.; Kiss, S.; Gasmi, M. Comprehensive Preclinical Assessment of ADVM-022, an Intravitreal Anti-VEGF Gene Therapy for the Treatment of Neovascular AMD and Diabetic Macular Edema. *J. Ocul. Pharmacol. Ther.* **2021**, *37*, 181–190. [CrossRef]
23. Rowe, L.W.; Ciulla, T.A. Gene Therapy for Non-Hereditary Retinal Disease: Age-Related Macular Degeneration, Diabetic Retinopathy, and Beyond. *Genes* **2024**, *15*, 720. [CrossRef]

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From Screening to Therapy: A Personalized Approach to ROP in a National NICU Setting

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Abstract: Aim: We aimed to investigate the incidence, treatment patterns, and associated risk factors of type 1 retinopathy of prematurity (ROP) in the only tertiary-level Neonatal Intensive Care Unit (NICU) in Cyprus. **Methods**: This retrospective study included all infants screened for ROP between January and December 2023. Data were collected from standardized NICU discharge summaries and included gestational age (GA), birth weight (BW), multiple birth, systemic infection, blood transfusion, oxygen therapy, surgical interventions, and ROP outcomes. Infants were categorized into non-ROP, non-type 1 ROP, and type 1 ROP groups. Statistical analysis was performed to identify differences in risk factor distribution. **Results**: Among 183 infants, 33 (18.0%) developed ROP, with 11 (6.0%) requiring treatment for type 1 ROP. All infants with type 1 ROP were born at ≤28 weeks GA and weighed <1501 g. Type 1 ROP was significantly associated with lower GA, lower BW, systemic infection, surgery, and prolonged oxygen support ($p < 0.05$). Six infants were treated with laser and three with intravitreal bevacizumab. No recurrence was observed in the anti-VEGF group during 18 months of follow-up. Two infants with aggressive ROP died before treatment. **Conclusions**: Type 1 ROP in Cyprus occurred exclusively in extremely preterm infants, associated with the cumulative effect of multiple risk factors. Laser remained the primary treatment, while anti-VEGF was used selectively with favorable outcomes. This study emphasizes the importance of tailoring ROP screening and treatment strategies based on individual neonatal risk profiles, supporting a personalized approach to neonatal ophthalmic care.

Keywords: type 1 ROP; Cyprus; anti-VEGF; laser photocoagulation; prematurity; neonatal risk factors; ROP screening

1. Introduction

Retinopathy of prematurity (ROP) is a worldwide leading cause of childhood blindness, resulting from disrupted retinal vascular development in preterm infants. As neonatal care advances globally, the survival of extremely premature infants has improved, yet this has also contributed to a rising incidence of severe forms of ROP. The pathophysiology of ROP involves two distinct phases. In the first phase, hyperoxia due to preterm birth suppresses vascular endothelial growth factor (VEGF), halting retinal vessel growth. The subsequent hypoxic phase leads to VEGF overexpression, triggering abnormal fibrovascular proliferation and potentially progressing to tractional retinal detachment if untreated [1,2].

Type 1 ROP represents the threshold at which treatment is clinically indicated and is defined by the Early Treatment for ROP (ETROP) criteria as stage 3 ROP in zone I or II with plus disease, or any stage ROP in zone I with plus disease. This classification reflects an aggressive disease course with a high risk of progression, requiring prompt intervention. Management strategies for type 1 ROP have evolved significantly in recent decades. Laser photocoagulation, once the mainstay of treatment, ablates avascular peripheral retina to reduce VEGF production and stabilize neovascularization. However, this technique compromises peripheral visual fields and increases the risk of high myopia [3].

In recent years, anti-VEGF agents like bevacizumab, ranibizumab, and aflibercept have emerged as alternatives or adjuncts to laser therapy. These agents target VEGF more selectively, allowing for continued peripheral retinal vascularization and resulting in less refractive error. Studies demonstrate their efficacy, particularly for aggressive ROP (A-ROP), though concerns remain about potential systemic effects due to VEGF's role in organogenesis and neurodevelopment [2,4,5].

Despite these advancements, the management of type 1 ROP remains complex, influenced by disease severity, anatomical location, and patient-specific factors. Both modalities have demonstrated efficacy, but they carry unique risks and benefits. Laser therapy is associated with greater structural stability in the long term, whereas anti-VEGF agents require careful follow-up due to the risk of late reactivation [6,7].

In Cyprus, neonatal care is centralized in a single tertiary Neonatal Intensive Care Unit (NICU), providing a unique opportunity to examine national ROP trends. To date, no comprehensive epidemiological data exist on the incidence, risk factors, and treatment outcomes of type 1 ROP in this setting. This study addresses this gap by investigating the characteristics and therapeutic patterns of ROP in Cyprus, with a particular emphasis on infants who required treatment for type 1 disease. Our findings aim to inform future clinical guidelines and support the development of personalized screening strategies in similar healthcare environments.

There is a growing recognition that neonatal care, particularly in managing ROP, benefits significantly from personalized medicine. Individual variability in gestational maturity and systemic comorbidities necessitate a tailored approach to screening frequency and therapeutic interventions. This study contributes to the understanding of how personalized neonatal care can optimize outcomes in a real-world NICU setting.

2. Methods

2.1. Study Design and Data Collection

This study was a single-center, retrospective cohort study conducted at the NICU in Makarios Hospital. This study included all infants who underwent ROP screening between 1 January and 31 December 2023.

This centralized setting allowed us to capture a comprehensive national cohort of preterm infants at risk of ROP, minimizing selection bias and ensuring population-level relevance.

2.2. ROP Screening Criteria

ROP screening was conducted according to institutional guidelines based on the UK Royal College of Ophthalmologists and Royal College of Paediatrics and Child Health (2008) recommendations [8]. Although updated UK guidelines were released in 2022, our institution's screening protocol during the study period was still based on the 2008 recommendations, which were in active use throughout 2023. The inclusion criteria for screening were

- Gestational age (GA) at birth <32 weeks;
- Birth weight (BW) < 1501 g.

Additionally, neonatologists could refer infants with GA $\geq$ 32 weeks or BW $\geq$ 1501 g for screening based on clinical concerns.

All examinations were performed by a single experienced pediatric ophthalmologist (VC), ensuring consistency in the diagnostic classification and reducing inter-observer variability.

2.3. Data Collection

Data were extracted from standardized NICU discharge summaries and electronic medical records. These documents provided structured information on demographics, perinatal risk factors, systemic complications, and ROP screening outcomes.

Collected variables included the following:

- Gestational age (in weeks) and birth weight (in grams);
- Type of pregnancy (singleton vs. multiple birth);
- Ethnic background;
- Systemic infections (defined as laboratory-confirmed sepsis);
- Blood transfusions;
- Surgeries (under general anesthesia);
- Duration of oxygen therapy (total hours on supplemental oxygen via mechanical ventilation, CPAP, or nasal cannula);
- ROP outcomes, including zone, stage, presence of plus disease, and treatment modality.

To ensure consistency, two independent investigators extracted data, and discrepancies were resolved through consensus with a third reviewer.

2.4. Classification of ROP and Treatment Protocol

The ROP classification followed the International Classification of ROP (ICROP) and UK screening and treatment guidelines updated in 2022 [9]. Infants were divided into three categories based on the most severe form of ROP observed during the screening period:

- Non-ROP;
- Non-type 1 ROP (defined as the presence of any type of ROP not requiring treatment);
- Type 1 ROP (defined as per ETROP criteria: zone I any stage with plus, zone I stage 3, or zone II stage 2 or 3 with plus disease).

Treatment decisions were made based on the anatomical location and severity of disease:

Laser photocoagulation was the first-line treatment for zone II stage 2 or 3 with plus disease. Treatment was performed using transpupillary green 532 nm laser photocoagulation (Visulas) in a near-confluent pattern.

Intravitreal anti-VEGF injection (bevacizumab 0.625 mg/0.025 mL) was used selectively for aggressive ROP (A-ROP) in cases involving zone I.

No other anti-VEGF agents were available in Cyprus during the study period.

Follow-up after treatment was individualized, with increased frequency during the first 3 months and extended follow-up beyond 18 months for anti-VEGF-treated infants, consistent with UK and international recommendations.

2.5. Statistical Analysis

Numerical variables were expressed as means $\pm$ standard deviation (SD) and categorical variables as percentages. Categorical variables were created for gestational age at birth (GA): group with GA $\leq$ 31 weeks + 6 days and group with GA > 32 weeks.

The study participants were divided into three subgroups based on the outcome: infants diagnosed with non-ROP, non-type 1 ROP, and type 1 ROP.

The incidence and the course of ROP in different GA categories were described by descriptive statistics.

Chi-square or Fisher's exact test for categorical variables and one-way ANOVA for continuous variables were used to compare differences between the groups.

All analyses were conducted using Stata version 18 (StataCorp, College Station, TX 77845, USA). Statistical significance was defined as a p-value < 0.05.

2.6. Ethical Approval

The current study has been registered and was approved by the National Bioethics Committee in Cyprus (n. 2023.01.143), and it was conducted according to the principles of the Declaration of Helsinki. The data were collected anonymously.

3. Results

In total, 183 patients were included in this study with a mean gestational age (GA) of 30.2 ± 2.7 weeks and a mean birth weight (BW) of 1382.5 ± 423.0 g. The incidence of ROP was 18.0% (N = 33). At a mean postmenstrual age of 34.3 ± 2.3 weeks, 22 infants developed non-type 1 ROP (66.6%) and 11 (33.3%) developed type 1 ROP and required treatment bilaterally. Sadly, two infants with type 1 ROP passed away before receiving treatment.

In the three subcategories, the mean GA in weeks was 31.0 ± 2.1 for non-ROP, 27.2 ± 2.4 for non-type 1 ROP, and 25.4 ± 1.5 for type 1 ROP. Additionally, the mean BW in grams was 1493.5 ± 353.8 for non-ROP, 966.4 ± 372.9 for non-type 1 ROP, and 701.6 ± 47.0 for type 1 ROP (Table 1).

Table 1. Patients' characteristics and risk factors' distribution among non-ROP, non-type 1 ROP, and type 1 ROP.

Patients' Characteristics	Non-ROP N = 150	Non-Type 1 ROP N = 22	Type 1 ROP N = 11	p-Trend *
	n (%)	n (%)	n (%)	
Birth weight in grams (mean ± SD)	1493.5 ± 353.8	966.4 ± 372.9	701.6 ± 47.0	**<0.001**
Birth weight < 1501 g				
No	64 (42.7)	2 (9.1)	0 (0)	**<0.001**
Yes	86 (57.3)	20 (90.9)	11 (100)	
Gestational week (mean ± SD)	31.0 ± 2.1	27.2 ± 2.4	25.4 ± 1.5	**<0.001**
Gestational week ≤ 31 + 6 days				
No	70 (46.7)	1 (4.5)	0 (0)	<0.001
Yes	80 (53.3)	21 (95.5)	11 (100)	
Multiple births				
No	94 (82.5)	15 (68.2)	6 (54.5)	0.743
Yes	56 (82.4)	7 (31.8)	5 (45.5)	
Race				
African	13 (8.7)	1 (4.5)	1 (9.1)	0.804
Asian	9 (6.0)	2 (9.1)	1 (9.1)	
Caucasian	128 (85.3)	19 (86.4)	9 (81.8)	

Table 1. *Cont.*

Patients' Characteristics	Non-ROP N = 150	Non-Type 1 ROP N = 22	Type 1 ROP N = 11	*p*-Trend *
Infection				
No	96 (95.0)	4 (18.2)	1 (9.1)	**<0.001**
Yes	54 (65.9)	18 (81.8)	10 (90.9)	
Blood transfusion				
No	30 (96.8)	1 (4.5)	0 (0)	0.081
Yes	120 (79.0)	21 (95.5)	11 (100)	
Surgery				
No	141 (83.9)	19 (86.4)	8 (72.7)	**0.021**
Yes	9 (60.0)	3 (13.6)	3 (27.3)	
Oxygen support				
No	9 (100)	0 (0.0)	0 (0.0)	0.777
Yes	141 (81.0)	22 (94.0)	11 (100)	
Oxygen duration in hours (mean ± SD)	362.9 ± 380.3	1513.4 ± 743.9	1844.0 ± 1549.7	**<0.001**

* values in bold font indicate statistical significance ($p < 0.05$).

There was a statistical difference between the three groups for BW < 1501 g, GA ≤ 31 weeks + 6 days, infections, surgery, and prolonged use of oxygen ($p < 0.05$).

As Figure 1 illustrates, the incidence and severity of ROP decrease sharply with increasing gestational age, with treatment-requiring ROP (type 1 ROP) confined almost entirely to infants born at ≤28 weeks, with the highest proportion at 24 weeks, where it accounts for nearly 80.0% of cases.

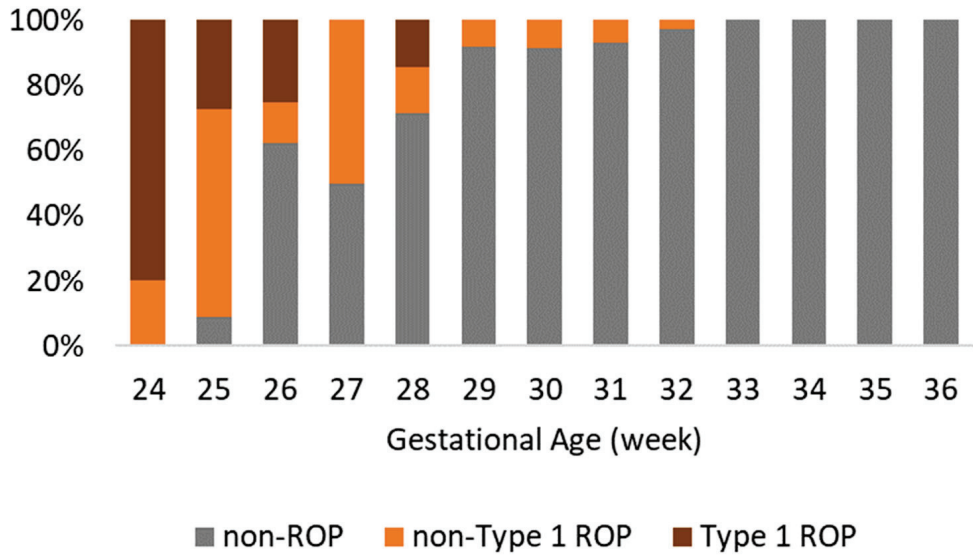

Figure 1. ROP severity trends across gestational ages at birth.

Among the eleven infants diagnosed with type 1 ROP, six received laser photocoagulation, for stage 3 with plus disease in zone 2, three were treated with intravitreal anti-VEGF injections for aggressive ROP, and two with aggressive ROP unfortunately passed away prior to receiving treatment.

For the laser-treated group, the mean postmenstrual age at ROP onset was 34.33 ± 1.03 weeks (range: 33–36 weeks), and the mean age at treatment was 41.00 ± 3.22 weeks (range:

37–46 weeks). Five cases had only one course of laser treatment, and one had additional laser treatment within 2 weeks from the first treatment.

The three infants who received anti-VEGF injections had a mean ROP onset at 34.00 ± 1.00 weeks (range: 33–35 weeks), and treatment was administered at a mean of 36.00 ± 0.00 weeks. The two deceased infants were diagnosed with aggressive posterior ROP at 34 and 38 weeks postmenstrual age, respectively. All three infants who received intravitreal bevacizumab injections showed no recurrence of ROP over the subsequent 18 months. Retinal vascularization was completed after several months, with a mean time of 8.17 ± 0.76 months.

Furthermore, the statistical difference amongst the three groups for BW, GA, and duration of oxygen support can be seen in Figure 2 (all p-values < 0.001).

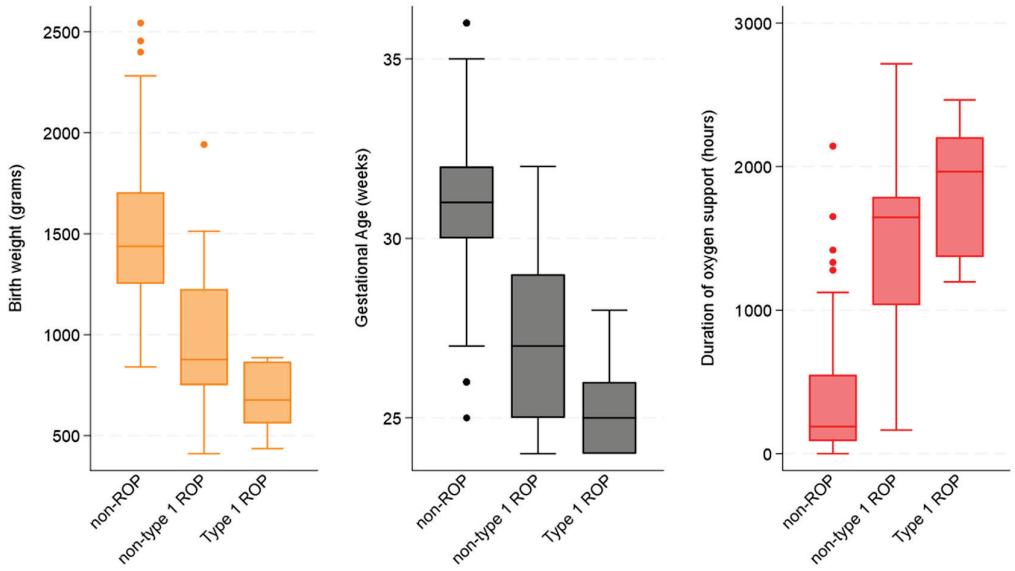

Figure 2. Distribution and comparison of birth weight (orange), gestational age (grey), and duration of oxygen support (red) by ROP category.

However, when non-type 1 ROP cases were compared with type 1 ROP cases, the only statistical difference was found for mean BW (966.4 ± 372.9 vs. 701.6 ± 47.0; $p = 0.032$) and mean GA (27.2 ± 2.4 vs. 25.4 ± 1.5; $p = 0.024$).

It is also noteworthy that, in our cohort, 12 infants had severe intrauterine growth retardation and low postnatal weight gain. Among them, three developed type 1 ROP and required treatment, while another four developed ROP but did not require treatment.

4. Discussion

This study offers valuable epidemiological data on type 1 ROP in Cyprus, focusing on the prevalence, treatment outcomes, and associated risk factors in the country's only tertiary NICU. Among 183 screened babies, the incidence of any ROP was 18.0% and of type 1 ROP was 6.0%. These figures align closely with those from developed nations such as the USA, Spain, and the UK. For instance, the G-ROP study reported a 12.4% treatment rate for type 1 ROP, while Spanish and UK studies documented treatment incidences of 14.6% and 13.8%, respectively [10–12].

Large-scale analyses confirm that almost all treatment-requiring ROP cases occur in the smallest and most premature infants. In the G-ROP cohort, only 0.7% of severe ROP cases were in infants with a birth weight >1251 g, supporting the selective but vigilant screening of high-risk neonates [12]. Our finding that all type 1 ROP cases occurred in

≤28-week infants mirrors other national datasets, where gestational age and birth weight remain the strongest predictors of severe disease [10].

In our study, two treatment modalities were utilized: laser photocoagulation and intravitreal bevacizumab. Laser photocoagulation, the standard treatment for stage 3 ROP in zones 2 and 3, was the predominant choice. Laser is a well-established treatment with a low complication rate and proven safety profile [13,14].

Intravitreal bevacizumab was reserved for aggressive ROP (A-ROP), a rapidly progressing condition. Bevacizumab-treated infants in our cohort demonstrated complete retinal vascularization without recurrence in a 18-month follow-up, similar to findings from Spain, where anti-VEGF therapy showed low recurrence rates and favorable outcomes [1,10,15]. In this one-year review, there was ratio of one case of intravitreal injection to two laser treatments.

Due to the risk of late ROP reactivation and serious visual consequences, extended and diligent follow-up is essential after anti-VEGF therapy. Many experts recommend monitoring infants beyond traditional screening timelines (frequent follow-up to 18 months after treatment and then 6-monthly or annually up to the age of 5 years according to UK ROP treatment guidelines), ensuring that reactivation is caught early before irreversible damage occurs. It is a quick procedure, and selected cases respond well with a high safety profile compared to laser. However, it significantly increases the follow-up workload. As infants grow older, it also becomes increasingly difficult to examine the far retinal periphery [13,14].

Recent studies provide interesting insights into treatment approaches. For instance, Sen et al. (2021) introduced the outcomes of combining anti-VEGF injection and laser photocoagulation in type 1 ROP, concluding that this strategy was effective and safe, potentially reducing the need for the strict long-term follow-up often required with monotherapy [16]. In parallel, Tung et al. (2024) provide additional longitudinal insights, reporting that while ranibizumab-treated eyes showed a myopic shift and longer axial lengths at age three compared with bevacizumab, these differences diminished by age six due to emmetropization [17]. This indicates that early biometric disparities may not translate into long-term refractive disadvantages, though vigilant refractive monitoring remains warranted [17]. Furthermore, a meta-analysis of neurodevelopmental outcomes reported no statistically significant increase in severe neurodevelopmental impairment after intravitreal bevacizumab compared with laser or untreated controls; however, it did reveal a small reduction in Bayley-III motor scores, underscoring the importance of long-term multidisciplinary follow-up [18]. Given the ongoing discussion regarding the systemic effects of anti-VEGF agents like bevacizumab, the inclusion of long-term neurodevelopmental follow-up would be valuable in future studies to assess potential implications on overall infant development.

In our study, risk factor analysis identified low gestational age, low birth weight, and prolonged oxygen therapy as significant contributors to ROP development and progression. These findings are consistent with international studies, which emphasize gestational age and birth weight as dominant risk factors. The G-ROP study reported that infants with gestational ages ≤24 weeks or birth weights ≤500 g had the highest odds of developing type 1 ROP [12], similarly to our cohort, of which the group comprising 24 weeks of gestational age infants has the highest risk of ROP type 1. Consistent with our results, Gaber et al. (2021) identified low gestational age, low birth weight, and prolonged oxygen supplementation as independent predictors of type 1 ROP [19]. Interestingly, this study found an overall incidence of ROP of 34.1%, with type 1 ROP occurring in 26.3% of infants, and additionally suggested apnea and thrombocytopenia as significant associated factors. These results reinforce the multifactorial nature of disease pathogenesis, where both hypoxic–ischemic and inflammatory pathways are implicated [19]. Importantly,

moving beyond these well-established clinical parameters, recent attention has shifted towards novel biomarkers. In particular, thrombocytopenia at birth (<181 $\times$ 10^9/L) and a characteristic pre-treatment platelet "spike" have been linked to an increased risk of type 1 ROP. Incorporating such laboratory markers into predictive algorithms could substantially enhance screening specificity and support the personalized scheduling of follow-up examinations [20].

Our findings further suggest that the cumulative presence of multiple risk factors exacerbates disease severity, particularly in cases of type 1 ROP. The Spanish study identified late-onset sepsis, mechanical ventilation, and weight at 28 days as independent predictors of ROP progression [10]. Other additional risk factors, for which their role needs to be elucidated further, are maternal pre-eclampsia and gestational diabetes [21], maternal age and emotional stress [22], premature rupture of membranes and chorioamnionitis, higher level of oxygen and fluctuation of oxygen levels, necrotizing enterocolitis, intraventricular hemorrhage, and poor postnatal weight. Some of these results seem to be contradictory in clinical studies due either to different and heterogenous preterm infant populations or different neonatal care across the world. In addition to biological and neonatal factors, broader determinants such as maternal health and socioeconomic status—although not included in our analysis—may also influence ROP development. These aspects, typically managed within neonatal care systems, warrant further investigation in future interdisciplinary research. This study has some limitations. Its retrospective design and relatively small sample size, especially for treated cases, limit the generalizability of the findings. The lack of long-term follow-up data, particularly for infants treated with anti-VEGF therapy, precludes definitive conclusions about treatment safety and durability. Nonetheless, this study establishes a strong foundation for understanding type 1 ROP trends in Cyprus and highlights areas for future research and clinical improvements. Moreover, future studies conducted under the updated 2022 UK guidelines may yield different screening outcomes, particularly with regard to inclusion criteria and follow-up recommendations.

5. Conclusions

This one-year review of type 1 retinopathy of prematurity in Cyprus provides the first national insight into the epidemiology, treatment patterns, and risk factors associated with type 1 ROP in a centralized tertiary NICU setting.

This study confirms that lower gestational age and birth weight are the strongest predictors of ROP severity, with type 1 ROP occurring exclusively in infants born at $\leq$28 weeks. Additional significant associations were found with systemic infections, prior surgeries, and the need for prolonged oxygen support. The findings also highlight the cumulative effect of multiple risk factors, reinforcing the importance of comprehensive neonatal care and early screening.

Laser photocoagulation remained the most frequently used treatment modality, while anti-VEGF was reserved for aggressive ROP cases. Both treatments were effective in halting disease progression, with no recurrence observed during an 18-month follow-up in the anti-VEGF group.

These findings support the ongoing need for tailored screening strategies, resource allocation, and long-term monitoring to optimize outcomes for preterm infants in Cyprus.

Our findings have several practical implications for neonatal ophthalmic care. Type 1 ROP developed exclusively in extremely preterm infants with additional systemic risk factors highlights the value of risk-adapted screening protocols beyond gestational age or birth weight alone. The favorable outcomes observed with both laser and anti-VEGF therapy support their continued use, with anti-VEGF reserved for selected cases where laser is less suitable. Given the extended follow-up required after anti-VEGF treatment,

structured post-treatment monitoring is essential. The recent approval of aflibercept for ROP treatment in Cyprus' General Health System as an alternative to bevacizumab introduces new opportunities for individualized treatment, outcome comparisons, and future clinical audits. Finally, the centralized care model in Cyprus offers a strong basis for developing a national ROP registry, which could support quality improvement and personalized screening strategies.

In conclusion, this study provides a comprehensive understanding of type 1 ROP in Cyprus, serving as a foundation for future research and local healthcare improvements.

This study underscores the relevance of personalized medicine in neonatal ophthalmology, advocating for risk-based screening and tailored treatment protocols to improve ROP outcomes in diverse populations.

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Institutional Review Board Statement: The current study has been registered and approved by the National Bioethics Committee in Cyprus (n. 2023.01.143, approved on 8 June 2023) and was conducted according to the principles of the Declaration of Helsinki. The data were anonymously collected from the discharged letters of the included patients.

Informed Consent Statement: Informed consent for participation was not required under local legislation, as this was a retrospective study using anonymized data (Cyprus Law 125(I)/2018, European GDPR Regulation (EU) 2016/679: https://eur-lex.europa.eu/eli/reg/2016/679/oj/eng (accessed on 13 August 2025)).

Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Wang, S.D.; Zhang, G.M.; Shenzhen Screening for Retinopathy of Prematurity Cooperative Group. Laser therapy versus intravitreal injection of anti-VEGF agents in monotherapy of ROP: A Meta-analysis. *Int. J. Ophthalmol.* **2020**, *13*, 806–815. [CrossRef] [PubMed]
2. VanderVeen, D.K.; Melia, M.; Yang, M.B.; Hutchinson, A.K.; Wilson, L.B.; Lambert, S.R. Anti-Vascular Endothelial Growth Factor Therapy for Primary Treatment of Type 1 Retinopathy of Prematurity. *Ophthalmology* **2017**, *124*, 619–633. [CrossRef] [PubMed]
3. Tsiropoulos, G.N.; Seliniotaki, A.K.; Haidich, A.B.; Ziakas, N.; Mataftsi, A. Comparison of adverse events between intravitreal anti-VEGF and laser photocoagulation for treatment-requiring retinopathy of prematurity: A systematic review. *Int. Ophthalmol.* **2022**, *43*, 1027–1062. [CrossRef] [PubMed]
4. Taher, N.O.; Ghaddaf, A.A.; Al-Ghamdi, S.A.; Homsi, J.J.; Al-Harbi, B.J.; Alomari, L.K.; Almarzouki, H.S. Intravitreal Anti-vascular Endothelial Growth Factor Injection for Retinopathy of Prematurity: A Systematic Review and Meta-Analysis. *Front. Med.* **2022**, *9*, 884608. [CrossRef] [PubMed]
5. Wiecek, E.; Akula, J.D.; Vanderveen, D.K.; Mantagos, I.S.; Wu, C.; Curran, A.L.; De Bruyn, H.; Peterson, B.; Fulton, A.B. Longitudinal Change of Refractive Error in Retinopathy of Prematurity Treated with Intravitreal Bevacizumab or Laser Photocoagulation. *Am. J. Ophthalmol.* **2022**, *240*, 252–259. [CrossRef] [PubMed]
6. Zhang, D.L.; Yoon, H.H.; Raye-Ann, O.D.; Arzu, J.; Rahmani, S. Postnatal Growth Trajectories and Neurodevelopmental Outcomes Following Bevacizumab Treatment for Retinopathy of Prematurity. *Clin. Ophthalmol.* **2022**, *16*, 2713–2722. [CrossRef] [PubMed]
7. Tan, H.; Blasco, P.; Lewis, T.; Ostmo, S.; Chiang, M.F.; Campbell, J.P. Neurodevelopmental outcomes in preterm infants with retinopathy of prematurity. *Surv. Ophthalmol.* **2021**, *66*, 877–891. [CrossRef] [PubMed]
8. Royal College of Paediatrics and Child Health, Royal College of Ophthalmologists & British Association of Perinatal Medicine. 2008. Available online: https://curriculum.rcophth.ac.uk/wp-content/uploads/2014/12/2008-SCI-021-Guidelines-Retinopathy-of-Prematurity.pdf (accessed on 13 August 2025).

9. The Royal College of Ophthalmologists. Treating Retinopathy of Prematurity in the UK. Clinical Guidelines. March 2022. Available online: https://www.rcophth.ac.uk (accessed on 13 August 2025).
10. de las Rivas Ramírez, N.; Luque Aranda, G.; Rius Diaz, F.; Perez Frias, F.J.; Sánchez Tamayo, T. Risk factors associated with Retinopathy of Prematurity development and progression. *Sci. Rep.* **2022**, *12*, 21977. [CrossRef] [PubMed]
11. Tavassoli, S.; Wach, R.; Haynes, R.; Markham, R.; Williams, C. Estimate of incidence of ROP requiring treatment in extreme preterms and impact on service—7 year review in tertiary unit. *Eye* **2019**, *33*, 845–849. [CrossRef] [PubMed]
12. Ying, G.S.; Bell, E.F.; Donohue, P.; Tomlinson, L.A.; Binenbaum, G.; G-ROP research group. Perinatal Risk Factors for the Retinopathy of Prematurity in Postnatal Growth and Rop Study. *Ophthalmic Epidemiol.* **2019**, *26*, 270–278. [CrossRef] [PubMed]
13. Yasin, A.; Sinha, S.; Smith, R.; Jain, S.F.; Hejkal, T.; Rychwalski, P. Reactivation of retinopathy of prematurity six years after intravitreal injection of bevacizumab. *J. Am. Assoc. Pediatr. Ophthalmol. Strabismus* **2023**, *27*, 236–239. [CrossRef] [PubMed]
14. Taylor, K.; Ghergherehchi, L.; Rao, P.; Harper III, C.A.; Chang, E. Very late-onset reactivation of retinopathy of prematurity post anti-VEGF bevacizumab treatment for type 1 ROP: A case report. *J. Am. Assoc. Pediatr. Ophthalmol. Strabismus* **2021**, *25*, 180–184. [CrossRef] [PubMed]
15. Ortiz-Seller, A.; Martorell, P.; Barranco, H.; Pascual-Camps, I.; Morcillo, E.; Ortiz, J.L. Comparison of different agents and doses of anti-vascular endothelial growth factors (aflibercept, bevacizumab, conbercept, ranibizumab) versus laser for retinopathy of prematurity: A network meta-analysis. *Surv. Ophthalmol.* **2024**, *69*, 585–605. [CrossRef] [PubMed]
16. Sen, P.; Agarwal, A.A.K.; Bhende, P.; Ganesan, S. Treatment outcomes of combination of anti-vascular endothelial growth factor injection and laser photocoagulation in Type 1 ROP and APROP. *Int. Ophthalmol.* **2022**, *42*, 95–101. [CrossRef] [PubMed]
17. Tung, H.F.; Chen, Y.L.; Chen, Y.C.; Chiu, S.L.; Chen, S.N. Six years follow-up of type 1 retinopathy of prematurity (ROP) treated with intravitreal injection of ranibizumab and bevacizumab: Long-Term Outcomes of intravitreal injection in Type 1 ROP. *Medicine* **2024**, *103*, e39251. [CrossRef] [PubMed]
18. Tsai, C.Y.; Yeh, P.T.; Tsao, P.N.; Chung, Y.C.E.; Chang, Y.S.; Lai, T.T. Neurodevelopmental Outcomes after Bevacizumab Treatment for Retinopathy of Prematurity. *Ophthalmology* **2021**, *128*, 877–888. [CrossRef] [PubMed]
19. Gaber, R.; Sorour, O.A.; Sharaf, A.F.; Saad, H.A. Incidence and Risk Factors for Retinopathy of Prematurity (ROP) in Biggest Neonatal Intensive Care Unit in Itay Elbaroud City, Behera Province, Egypt. *Clin. Ophthalmol.* **2021**, *15*, 3467–3471. [CrossRef] [PubMed]
20. Parrozzani, R.; Marchione, G.; Fantin, A.; Frizziero, L.; Salvadori, S.; Nardo, D.; Midena, G. Thrombocytopenia as Type 1 ROP Biomarker: A Longitudinal Study. *J. Pers. Med.* **2021**, *11*, 1120. [CrossRef] [PubMed]
21. Kim, S.J.; Port, A.D.; Swan, R.; Campbell, J.P.; Chan, R.P.; Chiang, M.F. Retinopathy of prematurity: A review of risk factors and their clinical significance. *Surv. Ophthalmol.* **2018**, *63*, 618–637. [CrossRef] [PubMed]
22. Stylianou-Riga, P.; Kouis, P.; Kinni, P.; Rigas, A.; Papadouri, T.; Yiallouros, P.K.; Theodorou, M. Maternal socioeconomic factors and the risk of premature birth and low birth weight in Cyprus: A case–control study. *Reprod Health* **2018**, *15*, 157. [CrossRef] [PubMed]

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Two Machine Learning Models to Economize Glaucoma Screening Programs: An Approach Based on Neural Nets

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Abstract: Background: In glaucoma screening programs, a large proportion of patients remain free of open-angle glaucoma (OAG) or have no need of intraocular eye pressure (IOP)-lowering therapy within 10 years of follow-up. Is it possible to identify a large proportion of patients already at the initial examination and, thus, to safely exclude them already at this point? **Methods**: A total of 6889 subjects received a complete ophthalmological examination, including objective optic nerve head and quantitative disc measurements at the initial examination, and after an average follow-up period of 11.1 years, complete data were available of 585 individuals. Two neural network models were trained and extensively tested. To allow the models to refuse to make a prediction in doubtful cases, a reject option was included. **Results**: A prediction for the first endpoint, 'remaining OAG-free and no IOP-lowering therapy within 10 years', was rejected in 57% of cases, and in the remaining cases (43%), 253/253 (=100%) received a correct prediction. The second prediction model for the second endpoint 'remaining OAG-free within 10 years' refused to make a prediction for 46.4% of all subjects. In the remaining cases (53.6%), 271/271 (=100%) were correctly predicted. **Conclusions**: Most importantly, no eye was predicted false-negatively or false-positively. Overall, 43% all eyes can safely be excluded from a glaucoma screening program for up to 10 years to be certain that the eye remains OAG-free and will not need IOP-lowering therapy. The corresponding model significantly reduces the screening performed by and work load of ophthalmologists. In the future, better predictors and models may increase the number of patients with a safe prediction, further economizing time and healthcare budgets in glaucoma screening.

Keywords: glaucoma; machine learning; neural network; screening; prediction

1. Introduction

Glaucoma is a leading cause of irreversible blindness [1]. Worldwide, it is estimated that about 66.8 million people have visual impairment caused by glaucoma, with 6.7 million suffering from blindness. Intraocular pressure (IOP) is the most important—because it is modifiable—risk factor for the development and progression of this disease [2]. Glaucoma is mostly asymptomatic until late in its course, when visual problems arise [3]. However, with early treatment, it is often possible to protect the eyes against serious vision loss [2]. Therefore, early diagnosis should be targeted. Due to the lack of symptoms in the early disease stage of glaucoma in most patients, this can only be achieved through screening programs. The most common subgroup of glaucoma in Europe is open-angle glaucoma

(OAG). It is well known in glaucoma research that age, IOP, PEX, and topographic disc parameter are univariable risk factors for predicting OAG.

A major goal in glaucoma research is to combine these risk factors in a multivariable fashion and to make predictions on a personalized level. Machine learning algorithms such as neural networks can personalize diagnosis for patients on an individual level, although several issues remain unresolved [4–6]. Because no such tools are currently in use that predict 10-year occurrence of OAG, we investigated to what extent a reliable prediction is feasible.

Several univariable risk factors for developing OAG are well known, such as age [4], increased IOP [2], PEX [7–9], and topographic disc parameters such the cup-to-disc ratio (C/D) [10,11]. Increased age, IOP, and cup-to-disc ratio, as well as PEX, are positively related to an increased risk of OAG. For example, age is an important risk factor for developing OAG; however, it is still unclear precisely how it affects the progression of OAG over time, particularly in relation to and in combination with other risk factors. Even if all these univariable risk factors are identifiable through a single examination at one point in time, it is difficult to diagnose OAG in certain cases, so why should we even consider attempting to make a prediction over a long period of 10 years?

There are mainly three reasons why models with a reject option should be used: (1) It is well known that patients with no or a small number of risk factors are highly likely to remain OAG-free over a long period. For example, subjects aged $\leq$ 50 years old with an IOP $\leq$ 18mmHg and a cup-to-disc ratio (C/D) $\leq$ 0.2 are highly likely to remain OAG-free over many years [3,10,11]. Thus, the basic idea is to identify a—hopefully as large as possible—subpopulation at the initial examination that will remain OAG-free at the 10-year follow-up point. (2) Studies have demonstrated that 10-year OAG incidence is quite low, ranging from 0.7 to 2.2% [12,13]. Hence, most patients remain OAG-free over 10 years, which makes predicting OAG freeness easier compared to incidences around 50%. (3) The third, and by far the most important, reason is related to the so called 'reject option' of the model. It is definitely not necessary to identify every single eye that remains stable. Usually, one expects that a model will make a prediction for every single patient. However, to reduce the screening time, it is sufficient to identify a preferably large subpopulation of patients with eyes that remain stable. If the prediction is too difficult and the accuracy of the prediction model is 'in doubt', it is still better to refuse to make a prediction instead of making a false-positive or false-negative prediction. Patients who do not receive a prediction are sent to the glaucoma specialist as usual. This so-called 'reject option' is crucial and a highly desirable advantage, because the models are not 'forced to make a prediction'. In other words, if the model has enough evidence, then it makes a prediction; otherwise, it refuses to make a prediction and asks the glaucoma expert to make the final decision (or to make no prediction at all). There is a cost to developing this model, because we have to accept that a large number of eyes do not receive a prediction. On the other hand, the reject option substantially increases the accuracy of the model for those patients who receive a prediction. This allows for identifying a large number of patients for whom the corresponding eye can safely be excluded, and, thus, much time and effort in screening studies can be saved. All definitions, endpoints, methods, and results refer to right eyes, as machine learning algorithms are expected to clearly distinguish between right and left eyes. New models for left eyes can be trained and tested using the same methods.

This study will answer the following questions by considering the principle of very high accuracy: (1) Which of these endpoints shows reasonable results? (2) How good are the performances of the neural network models in terms of accuracy? How many patients receive a false-negative or false-positive prediction? (3) To what extent do these models help to reduce daily work load, i.e., how many eyes receive a prediction, and in how many cases is the algorithm in doubt? (4) How can these approaches be improved in the future?

2. Materials and Methods

The Salzburg-Moorfields-Collaborative-Glaucoma Study (SMCGS) began in 1996 and is part of a large 25-year glaucoma blindness prevention program in Austria, Europe. The SMCGS is a retrospective monocentric population-based longitudinal cohort study. This study mainly provides information for and identifies glaucoma suspects, offering timely and adequate treatment in clear-cut cases of glaucoma.

2.1. Definition of Definite OAG

We defined glaucoma as a progressive optic neuropathy leading to the loss of retinal nerve fibers and glia cells, with characteristic alterations on the optic nerve head and with or without corresponding functional alterations in visual field examination [14]. The following parameters were used here: (a) Optic nerve head (ONH)—notching, rimming, arbitrary chosen subjective cup-to-disk (C/D) ratio of 0.45 or more, or C/D asymmetry between the two eyes >0.3 not explainable through other diseases. (b) Visual field—glaucomatous visual field defects corresponding to optic disc changes. (c) Open anterior chamber angle [14]. Inclusion criteria: age $\geq$ 40 years, no OAG or other type of glaucoma, best spectacle-corrected visual acuity >6/9, refractive range ranging from -6.00 to +4.00 diopters with a difference in refraction between both eyes <3.00 diopters. Exclusion criteria: pseudophakia, IOP-lowering therapy at the initial examination, and eye diseases potentially leading to visual field defects or secondary increases in intraocular pressure, contraindication against beta blockers, systemic corticosteroid therapy, or pregnancy.

During the study's duration of more than 27 years, up to 24 ophthalmologists and 7 ophthalmic assists specially trained in using the study protocol performed ophthalmic examinations and graded each patient. SMCGS data collection started on the 1st December 1996. Baseline: The ophthalmic assistants were responsible for oral education and SMCGS, questionnaire interviews, best corrected visual acuity (BCVA) measurements with the Early Treatment Diabetic Retinopathy Study (EDTRS) chart, subjective refraction, visual field examination (VF; Motion-Sensitivity Test (MST), Humphrey Frequency Doubling Technology Perimetry (FDT, Humphrey FDT matrix, Humphrey Instruments Inc., San Leandro, CA, USA), Humphrey Field Analyzer II or II-I [HFA, Humphrey Instruments Inc., San Leandro, CA, USA]), central corneal thickness (CCT) with ultrasound pachymetry (Pocket II, Quantel Medical, Cournon d'Auvergne Cedex, France) and structural imaging (confocal scanning laser ophthalmoscopy was carried out using TopSS® (Fa. LDT, San Diego, CA, USA)), which refers to a scanning laser tomography technique for analyzing the eye, specifically the optic nerve head. This method uses scanning laser tomography to depict the top surface of the optic nerve, allowing for detailed analysis of structural changes. Subsequently, all subjects underwent a standardized eye examination with intraocular pressure (IOP) assessment in miosis and mydriasis through Goldmann applanation tonometry (Haag Streit, Koeniz, Switzerland), gonioscopy (to exclude angle-closure), slit lamp anterior segment examination, and dilated fundus slit lamp biomicroscopy for optic disc assessment using a Volk 90 D lens, including subjective C/D. PEX was diagnosed during the initial clinical examination in the glaucoma screening ambulance using a slit lamp. Recorded TopSS® values included the C/D ratio, total contour area, effective area, neuroretinal rim area, half-depth area, half-depth volume, and volume below. If results were abnormal or inconclusive during the VF-examination at baseline, a second confirmation test was performed for clarification.

2.2. Definition of Endpoints

We defined two different endpoints: E1—composite endpoint, i.e., freeness of OAG and no IOP-lowering therapy; E2: freeness of OAG (with or without IOP-lowering therapy).

If a patient (1) still wants to join the screening program, (2) agrees to receive IOP-lowering therapy if necessary, and (3) is only interested in determining whether OAG will develop, then E2 is best. E1 is suitable for informing and safely excluding the patient from a screening program. Here, we want to show that for E1 and E2, ocular hypertension (OHT) might develop (in the case of E2, with a possibly necessary IOP-lowering treatment), but OAG will not develop.

Age, IOP [2], PEX [7–9], and 8 optic nerve head parameters (TopSS®) were used as continuous predictors for E1 and E2.

2.2.1. Years Follow-Up

Patients free of glaucoma (OAG or another type of glaucoma) at the baseline visit received follow-up only. All examinations were carried out the same manner as at the baseline examination. According to the results of the baseline examination, patients were also scheduled for interim visits in the first decade. Complete data for the baseline visit and the 10-year visit were used to train and validate the machine learning models.

2.3. Statistical Methods and Model Development

Data were cleaned for inaccurate or missing data, resulting in data for 585 patients, with full records of each predictor and the outcome variable. Univariable logistic regression models were used to estimate the odds ratios of each individual predictor with 95% confidence intervals.

2.4. Machine Learning Algorithms, Training and Testing

Multilayer perceptron neural networks, nearest-neighbor classifiers, Bayes classifiers, random forest models, logistic regression models, and support vector machines were trained and tested.

2.5. Data for Model Training and Testing

The full sample (n = 585) was randomly split into a training sample (n = 486) and a test sample (n = 117). No data from the test sample were used for model training. All neural networks were first trained and 10-fold cross-validated in a training sample (n = 468) and finally tested in an independent randomly selected test sample (n = 117) to ensure generalization to new, previously unseen eyes.

2.6. Methods for Model Training

2.6.1. Neural Nets

Adaptive moment estimation was used as a stochastic gradient descent method. To mitigate overfitting, an early stopping approach and 10-fold cross-validation were used. L2-regularization techniques were also used. Additionally, early stopping, batch normalization layers, and L2-regularization techniques were used. The batch size was 64 and the maximal number of training rounds was 5000 (Table S1). The network architecture is illustrated in Figure S1.

2.6.2. Other Models

All other models were trained according to the default settings of MATHEMATICA [15].

2.7. Feature Selection

Potential risk factors were selected as input variables, which are listed in Table 1. Although some of these risk factors were not significant, after applying a genetic algorithm and the reject option (as described in the next subsection), these predictors were still useful for limiting the number of rejected cases.

Table 1. Univariable odds ratios and descriptive statistics of various predictors for composite endpoint E1: freeness of. OAG and having no IOP-lowering therapy within 10 yrs.

	OAG-Free and no IOP Lowering Therapy 10 yr FU (n = 544)		OAG or IOP-Lowering Therapy at 10 yr FU (n = 41)			
	Mean	**Std**	**Mean**	**Std**	**Odds Ratio**	***p*-Value**
Age (yrs)	58.84	7.96	60.7	7.29	1.02 (0.99–1.08)	0.16 [1]
IOP (mmHg)	15.1	2.83	18.5	4.27	1.33 (1.21–1.46)	<0.0001 [1,*]
PEX	12/532	2.2%	3/38	7.3%	3.5 (0.6–13.7)	0.027 [2,*]
Effective Area (mm^2)	0.95	0.40	1.08	0.32	2.37 (1.02–5.5)	0.042 [1,*]
Neuroretinal Rim Area (mm^2)	1.29	0.32	1.19	0.18	0.38 (0.13–1.09)	0.07 [1]
Half Depth Area (mm^2)	0.37	0.21	0.44	0.20	4.0 (1.01–15.8)	0.047 [1,*]
Half Depth Volume (mm^3)	−0.06	0.06	−0.07	0.05	0.08 (0.008–7.09)	0.26 [1]
Volume Below (mm^3)	−0.25	0.18	−0.31	0.20	0.23 (0.05–0.996)	0.047 [1,*]
Cup-To-Disc Ratio/0.1 unit change	0.42	0.14	0.48	0.12	1.46 (1.11–1.92)	0.006 [1,*]

[1] Independent t-test [2] Fisher's Exact test (one sided), odds ratios are based on univariable logistic regression models, *: indicates statistical significance.

2.8. Reject Option

From a clinical point of view, most importantly, we wanted to avoid making false-negative predictions for both endpoints. This means that E2 would predict that the patient will not suffer from OAG within 10 years, even though they may develop OAG within this period. For E2, a false-negative prediction may lead to irreversible eye damage or even blindness. For E1, the same scenario would occur, and no medication would be administered, which could also result in OAG or blindness. The predictors, however, did not have enough predictive power to achieve this goal for the whole cohort. Thus, it was reasonable to allow the algorithms to apply a 'reject option' in doubtful cases [16]. This means that 2 cut-offs for the posterior probabilities, instead of 1 cut-off, were first chosen in the training sample and then applied and tested in the test sample. Finally, to assess model performances, the percentages of predicted subjects and total correct predicted cases were computed in the training and test samples and compared to determine whether the networks generalized well to new, previously unseen data.

The advantage of this approach was that it minimized the number of false-negative and false-positive predictions. This important advantage came at a cost, as a large number of cases did not receive a prediction. We recommend that patients who do not receive a prediction should still join a glaucoma screening program to benefit from expert medical knowledge.

All analyses were carried out using STATISTICA 13 [17] and MATHEMATICA 13, developed by Wolfram Research, Inc., Champaign, IL (2023) [15].

3. Results

In the original screening study, 6889 subjects were screened and 976 subjects were examined ten years later. Finally, after applying all inclusion and exclusion criteria and removing all missing data, 585 right eyes with complete data were included.

3.1. Demographic Data at Baseline

An overview is given in (Table 2).

Table 2. Overview of baseline demographic data of n = 585 subjects with complete data at baseline and 10 year follow-up [1].

	Mean	SD	−95% CI	+95% CI
Age (y)	58.9	8.1	58.8	59.6
Follow-up (years)	11.1	1.1	10.99	11.2
IOP (mmHg)	15.3	3.2	15.1	15.6
Total Contour Area (mm^2)	2.23	0.44	2.20	2.27
Effective Area (mm^2)	0.96	0.39	0.93	0.99
Neuroretinal Rim Area (mm^2)	1.28	0.33	1.25	1.31
Half Depth Area (mm^2)	0.38	0.21	0.36	0.40
Half Depth Volume (mm^3)	−0.06	0.057	−0.066	−0.057
Volume Below (mm^3)	−0.26	0.19	−0.27	−0.24
Cup-To-Disc Ratio	0.42	0.14	0.41	0.43
	N	**Percentage**	**−95% CI**	**95% CI**
Endpoint E1: OAG [2] at 10-year FU or IOP lowering therapy within 10 years	41/585	7%	5.1%	9.4%
Endpoint E2: OAG [2] at 10-year FU	21/585	3.6%	2.23%	5.43%

[1] All results refer to right eyes. [2] OAGs included 6 primary open angle glaucoma (1.03%), 3 pseudoexfoliation glaucoma (0.51%), 0 pigmentary glaucoma (0%), and 12 normal tension glaucoma (2.05%).

3.2. Overview of Demographic Data at 10-Year Follow-Up

Within our dataset, OAGs included 6 primary open-angle glaucoma (1.03%) cases, 3 pseudoexfoliation glaucoma (0.51%) cases, 0 pigmentary glaucoma (0%) cases, and 12 normal tension glaucoma (2.05%) cases. A total of 41 of 585 eyes had OAG or received at least one or more types of IOP-lowering eye therapy within 10 years (E1), corresponding to an average 10-year incidence of 7% (5.1–9.4%). Differences between those who fulfilled E1 or not and the corresponding univariable prediction power (odds ratios) levels of possible candidate predictors are given in Table 1.

An overview of the performance accuracies of the machine learning models is given in Table 3, while the advantages and disadvantages of using the selected neural network models with both E1 and E2 are given in Table 4. The neural network models had the smallest percentage of unpredicted patients compared to the classical machine learning algorithm. An overview of the model performance with various combinations of cut-offs for endpoint E1 is given in Table S2. The logistic regression model (classical approach) also achieved 100% NPV; however, the number of unpredicted patients was considerably larger at 69.2%. The models M1 and M2 demonstrated excellent results, with a sufficiently large proportion of eyes receiving a prediction. Most importantly, no eye was predicted false-negatively or false-positively.

Table 3. Results of six machine learning algorithms to predict endpoint E1 based on accuracy. Results are given before application of the reject option and are based on the 10-fold cross-validation. Similar results were found for endpoint E2.

Model Performance	Support Vector Machine	Nearest Neighbors	Random Forest	Bayes Classifier	Neural Network
(NPV/PPV)%	93.2/92.3%	93.2/92.3%	93.2/92.4%	90.4/85.5%	93.5/93.2%

After application of the reject option, the best neuronal network model achieved a NPV of 100%, but rejected 314 of 585 (53.6%) patients in the overall sample and similar results were found for endpoint E2 (Table 4).

Table 4. Overview performance, advantages and disadvantages of two predictive neural network models with endpoints E1 [1] and E2 [2].

Endpoint	Percentage of Eyes Filtered Out/Received a Prediction (%)	Performances of the Models [3] (n/%)	Advantages	Disadvantages	Remarks
E1 [2]	57%/43%	253/253 (100%)	In total, 43% of all eyes can safely be excluded from a glaucoma screening program for up to 10 years if one wants to be certain that the eye remains OAG-free and will not require IOP-lowering therapy. This also significantly reduces the screening amount.	57% did not receive a prediction.	Eyes can safely be excluded from the OAG screening program, especially if the patient wants to leave the screening program.
E2 [1]	53.6%/46.4%	271/271 (100%)	Overall, 46.4% of all eyes can safely be excluded from a glaucoma screening program for up to 10 years if one wants to be certain that the eye remains OAG-free. This significantly reduces the screening workload of ophthalmologists.	Overall, 53.6% did not receive a prediction. Eyes receiving IOP-lowering therapy were not filtered out.	This model is designed for patients in a glaucoma screening setting within a hospital, assuming these patients want to remain within the screening program and continue with IOP-lowering therapy.

[1] Endpoint 1 (E1): remaining OAG-free within 10 years. [2] Endpoint 2 (E2) composite endpoint: remaining OAG-free + no IOP-lowering therapy within 10 years of FU. [3] Performances measured in the training and test sample based on neural networks.

3.3. Illustration of the Models Using Real Data

To better illustrate the models, typical data used in a glaucoma screening setting are provided at the initial examination, illustrating the predictions, decisions, and observations made by M1 for E1 (Table 5). On the left side, we see the predictors evaluated during the initial examination: age, IOP, effective area, neuroretinal rim area, volume below, half-depth area, half-depth volume, C/D, and PEX. On the right side, we see the predictions for M1 for E1 during the initial examination and corresponding observations of both endpoints made at the 10-year follow-up. Eyes highlighted in green were identified via the prediction model, and every single eye was correctly predicted. Eyes highlighted in gray did not receive a prediction, because the algorithm believed that their status was 'in doubt' and refused to make a prediction (i.e., the corresponding eye was 'filtered out'). For E1, 332 eyes (57%) were filtered out and did not receive a prediction, while 253 (43%) of all eyes received a prediction. In summary, all 253 eyes were correctly predicted (100%), meaning that no eye was predicted false-negatively or false-positively.

Table 5. Illustration of prediction model for endpoint E1 based on real data at the initial exam, predicted and observed endpoints at. 10-year follow-up. Identified eyes (green) received a prediction by the model and can safely be excluded from the screening program. Eyes without a prediction (gray) should be send to a glaucoma expert. Patients who suffered of OAG or needed IOP lowering therapy are highlighted in red. No predictions were done in these cases. 332 eyes (57%) were filtered out and did not receive a prediction, 253 (43%) of all eyes received a prediction. 253 out of 253 eyes were correctly predicted (100%). Most importantly, no eye was predicted false-negatively.

Initial Exam									Predictions of Endpoint E1 Made at Initial Exam	Observed Endpoint E1 at the 10 yr FU
Age	**IOP [1]**	**EA [2]**	**NR [3]**	**VB [4]**	**HDA [5]**	**HD [6]**	**C/D [7]**	**PEX [8]**		
62	12	0.85	1.22	−0.15	0.19	−0.02	0.41	No	No prediction	OAG-free/no IOP low. therapy
63	14	0.66	1.46	−0.15	0.24	−0.03	0.31	No	OAG-free/no IOP low. therapy	OAG-free/no IOP low. therapy
56	15	1.49	1.18	−0.35	0.60	−0.07	0.56	No	OAG-free/no IOP low. therapy	OAG-free/no IOP low. therapy
61	15	1.22	0.76	−0.52	0.48	−0.11	0.62	No	No prediction	OAG
66	14	1.41	1.10	−0.34	0.50	−0.09	0.56	No	OAG-free/no IOP low. therapy	OAG-free/no IOP low. therapy
42	17	0.51	1.73	−0.10	0.19	−0.03	0.23	No	OAG-free/no IOP low. therapy	OAG-free/no IOP low. therapy
54	14	0.85	0.91	−0.13	0.32	−0.02	0.49	No	No prediction	OAG
69	13	0.96	1.14	−0.18	0.39	−0.05	0.46	No	OAG-free/no IOP low. therapy	OAG-free/no IOP low. therapy
66	14	1.13	1.21	−0.31	0.34	−0.06	0.48	No	OAG-free/no IOP low. therapy	OAG-free/no IOP low. therapy
70	14	1.09	1.44	−0.17	0.27	−0.028	0.43	Yes	No prediction	IOP low. therapy
59	18	0.83	1.18	−0.21	0.22	−0.04	0.41	No	OAG-free/no IOP low. therapy	OAG-free/no IOP low. therapy
62	15	0.99	1.21	−0.45	0.39	−0.12	0.45	No	No prediction	OAG-free/no IOP low. therapy
57	12	0.50	1.39	−0.06	0.20	−0.01	0.27	No	OAG-free/no IOP low. therapy	OAG-free/no IOP low. therapy
58	13	1.16	0.97	−0.65	0.66	−0.22	0.54	No	No prediction	OAG-free/no IOP low. therapy
57	16	1.25	0.94	−0.41	0.69	−0.12	0.57	No	No prediction	OAG-free/no IOP low. therapy
62	12	1.21	1.25	−0.64	0.70	−0.19	0.49	No	No prediction	OAG-free/no IOP low. therapy

[1] Intraocular eye pressure (mmHg), [2] effective area (mm^2), [3] neuroretinal rim area (mm^2), [4] volume below (mm^3), [5] half depth area (mm^2), [6] half depth volume (mm^3), [7] cup-to-disc area, [8] pseudoexfoliation syndrome.

3.4. Illustration for the Need for a Reject Option

To better understand both why a large number of eyes were filtered out and the difficulty of predicting E1, we provide deeper insights how close both data clouds (E1, yes/no) closely stick together and illustrate the overlap between both data distributions (Figure 1). As indicated, there is a large overlap between both distributions, indicating the difficulty of identifying eyes for which accurate predictions can be made and eyes that must be rejected during the initial examination for making predictions for the future.

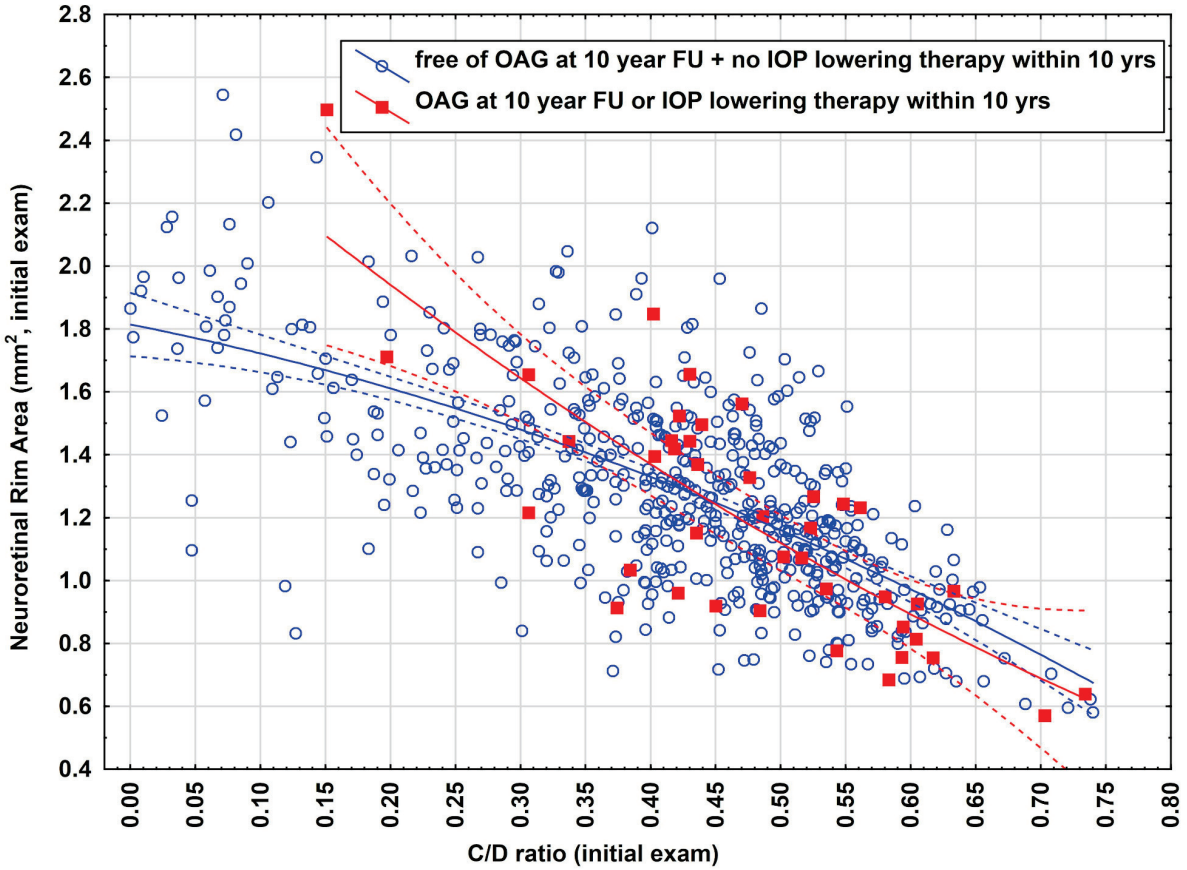

Figure 1. Scatterplot of C/D ratio and neuroretinal rim area (mm^2) of 585 eyes suggesting the need of a reject option. All eyes were OAG-free without receiving IOP-lowering eye therapy at the initial exam. Red dots indicate eyes fulfilling endpoint E1 (freeness of OAG + no IOP-lowering therapy within 10 years of FU) at the 10-year FU, blue dots indicate eyes which do not fulfill endpoint E1 at the 10 yr FU. Eyes with values in the left part of the figure ('low risk region') are very likely to remain OAG-free and having no IOP-lowering therapy within 10 years whilst eyes in the right region ('high risk region') are at high risk for developing a OAG or will receive an IOP-lowering therapy within 10 years of FU. It is precisely the main task of neural networks to identify regions of low risk in the space of all input variables (including age, IOP, optic nerve head parameters and PEX), simultaneously.

4. Discussion

This study provides a completely new and safe approach for identifying a large subpopulation of eyes remaining free of open-angle glaucoma without needing IOP-lowering therapy within 10 years. The most important benefit of M1 is that 43% of the eyes in the screening population could safely be excluded already at the initial examination, i.e., no predicted eye received a false-positive or false-negative prediction. It is important to emphasize that this accuracy could only be achieved by allowing the algorithm to refuse to make a prediction, occurring in 57% of eyes. Therefore, there is no need to further examine eyes that receive a OAG screening prediction within the next 10 years, saving corresponding time, effort, and costs. This implies that these eyes are no longer within a screening program and, thus, will not receive IOP-lowering therapy. This was a key point and crucial for explaining not only why E1 focuses on OAG but also why these eyes do not receive IOP-lowering therapy.

4.1. Discussion of Model M1

In this model, the patient will receive a positive response if they not only remain OAG-free over 10 years but also do not require IOP-lowering therapy, which is an important form of subjective relief for the person.

4.2. Discussion of Model M2

The patient receives a positive response if they remain OAG-free over 10 years. This model is the right choice for patients who are willing to remain in a glaucoma screening program. In this case, the eye may still receive IOP-lowering therapy if necessary. Therefore, E2 was defined as remaining OAG-free (with adequate IOP-lowering therapy if ocular hypertension arises). IOP-lowering therapy was not included compared to E2. A detailed overview of the clinical pathway used to guide the patient workflow is given in Figure 2.

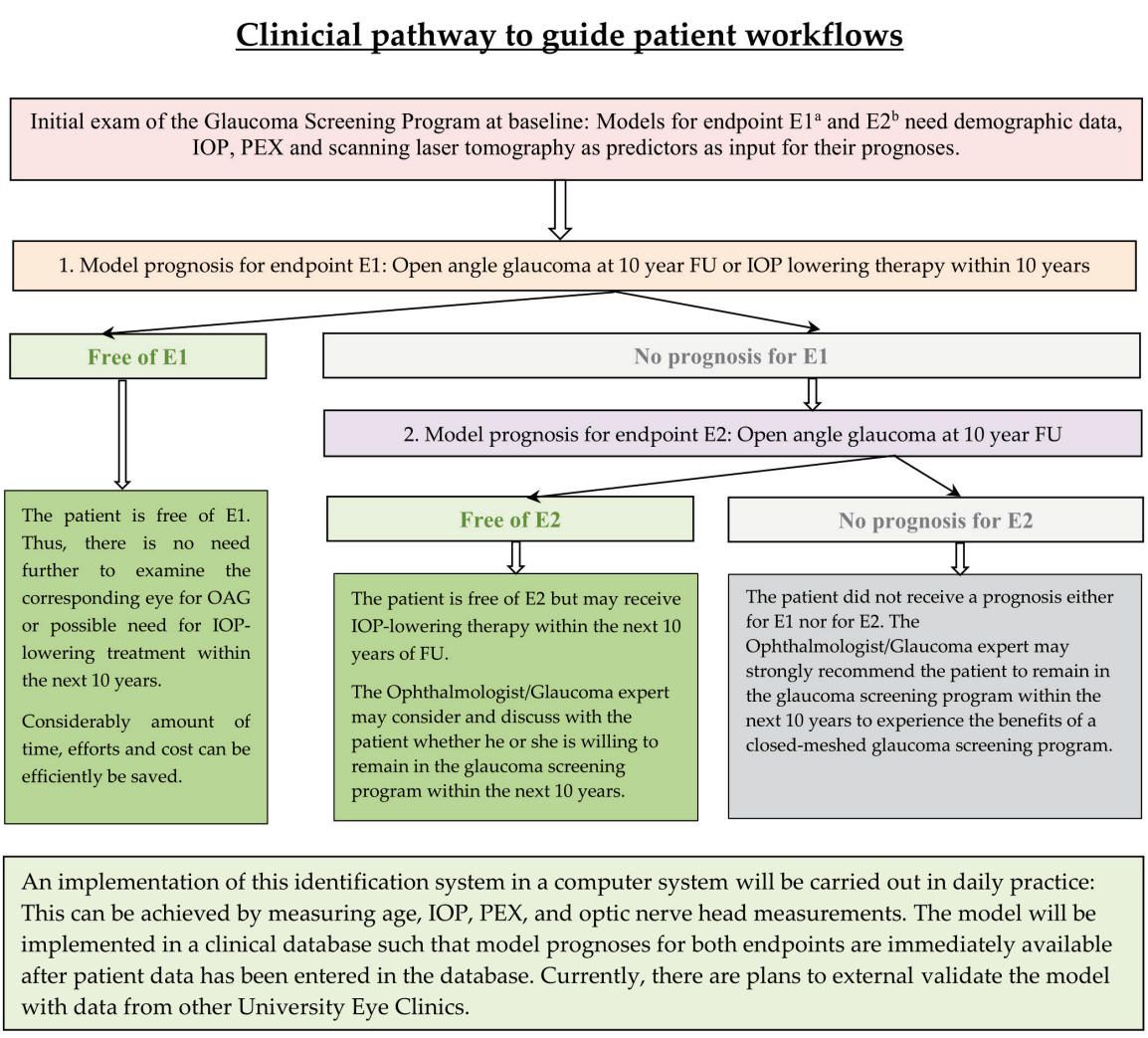

Figure 2. Clinicial pathway to guide patient workflows. a: Endpoint 1; b: Endpoint 2.

4.3. PEX as Risk Factor and How It Was Handled by the Models

Additionally, we analyzed how the models handled patients with PEX. There were 570 eyes without PEX, and 44% received a prediction. All predictions were correct. A total of 15 eyes had PEX, and the algorithms refused to make a prediction in all 15 eyes. This suggests that PEX is a risk factor that deserves special attention from glaucoma experts.

4.4. Implication for Practical Purposes

This identification system can be implemented in a computer system in daily practice: This can be carried out by measuring age, IOP, PEX, and optic nerve head measurements. The models can be implemented in a clinical database so that model prognoses for both endpoints are immediately available after patient data have been entered in the database. Currently, we plan to externally validate the model with other University Eye Clinics. Creating an open access form of the model is currently under discussion.

Of course, we do not screen for all known sight-threatening diseases but for one major cause of visual impairment. Therefore, we also consider personal responsibility and suggest that subjects should be screened anyway due to the risk of contracting other eye-threatening diseases. We suggest that the remaining patients without a prediction should be screened more closely.

4.5. Outlook and Further Developments

We have many ideas for further improving the models in the future: First, we plan to test and analyze more candidate predictor variables based on image data, e.g., GDx®, OCT®, HRT II®, or other structural imaging methods. Modern deep learning methods based on convolutional neural networks (CNNs), such as Inception net, VGGNet, ResNet, R-CNN+LSTM models, or classification maps, have already demonstrated utility for assessing various disease processes, including cataracts, glaucoma, age-related macular degeneration, and diabetic retinopathy [4,18–22]. Of course, these methods should also be applied, especially in combination with clinical data, for predicting the above-addressed endpoints.

Another option is to establish prediction models for subgroups of OAG or related diseases. Moreover, we could ask ourselves if we should increase or decrease the follow-up time (e.g., to 5 or 15 years). Here, we provide models for assessing the development of the most common sub-form of glaucoma (OAG), which is only one of the major reasons for the irreversible impairment of visual function and blindness worldwide. Applying our approach to the other subgroups of glaucoma and other major diseases causing irreversible impairment may reduce the burden of routine screenings tremendously.

4.6. Strengths and Limitations of This Study

4.6.1. Strengths

The most important strength of this study is that it provides methods for safely excluding 43% of all eyes during the initial examination and correctly predicting OAG freeness and freeness from IOP-lowering therapy for 10 years. This significantly decreases the practical screening workload in daily work. Another strength is that the models are not only theoretical concepts/ideas/suggestions, but they can also easily be implemented in any database and then be applied in daily practice. The large sample size of about 600 eyes is also an important strength of this study. A large sample size is important for creating with statistically sound models, as emphasized in mathematical statistics and machine learning theory. It is important to train and independently validate the models with large samples to avoid overlearning and to provide accurate predictions when the models are confronted with new, previously unseen data (generalization). Another strength of this study concerns the sample: right and left eyes were carefully distinguished and were not merged. This would artificially increase the sample size, but in that case, data independence may be undermined. In this case, prediction models may struggle to predict new, previously unseen data.

4.6.2. Limitations

There might be a possible imbalance between positive and negative groups in terms of predictive performance. This might be due to the fact that optic nerve head measurements were made using TopSS® (Fa. LDT, San Diego, CA, USA), which is outdated. We think that using predictors with higher predictive power (e.g., genetic markers) or using modern topographic measurements of the optic nerve head might improve predictions. Additionally, we recommend conducting follow-up studies with larger samples and evaluating the model with external independent data. However, for this study with such a long FU over 10 years, it was necessary to use this method to achieve a sufficiently large sample size. As described in the Materials and Methods section, visual field examinations were carried out using various different methods. In large-cohort studies such as ours, this is a common drawback over a long study period of more than 20 years, which implies that no uniform data source for the visual field was available. We strongly support using the above procedure for only examining cases where a shallow chamber is observed, as the vast majority of ophthalmologists in practice perform gonioscopy only in cases of a suspected configuration of the anterior segment. When comparing the results of different glaucoma screening programs, it is important to consider to which population the results finally refer. It is important to point out that data for these models were sampled from a European sample. It is well known that ethnicity and race are risk factors for glaucoma, and, therefore, we suggest applying these models in Caucasian patients only.

5. Conclusions

Overall, 43% of all eyes can safely be excluded from a glaucoma screening program for up to 10 years while remaining certain that the eyes remain OAG-free and will not need IOP-lowering therapy. We suggest applying this model to inform patients of their future eye health 10 years ahead and to safely exclude the identified eye from further screening within this period. This has a positive impact on reducing the enormous working and screening workloads of ophthalmologists. We suggest applying these neural network models for screening purposes to decide which eyes can be safely excluded and which eyes should be followed-up more closely and sent to a glaucoma expert. This filtering system and identification method will reduce the time and healthcare budgets required for glaucoma screening in the future.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jpm15080361/s1, Figure S1: An illustration of the network architecture with all layers and activation functions; Table S1: An overview about pre-processing, model training, split of data into training, validation and test sample, training stop criteria, selection of thresholds for the reject option and final test of the models based on neural networks; Table S2: Overview of model performances with various combinations of cut-offs for the neural network model with endpoint E1.

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Institutional Review Board Statement: The study was supported and approved by the Health administration council of the Federal State of Salzburg and conforms to the provisions of the Declaration of Helsinki in 1995. The study was approved by a local ethic committee (No 1120/2024). This study follows the CONSORT-AI Extension guidelines and the SPIRIT-AI guidelines.

Informed Consent Statement: Patient consent was waived by the Ethics Committee due to the retrospective nature.

Data Availability Statement: Data are available on request due to legal restrictions.

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References

1. Vision Loss Expert Group of the Global Burden of Disease Study; GBD 2019 Blindness and Vision Impairment Collaborators. Prevalence of Vision Loss in High-Income Countries and in Eastern and Central Europe in 2020: Magnitude and Temporal Trends. *Ophthalmic Epidemiol.* **2025**, 1–14, *ahead of print*.
2. Johns, K.L.; Faralli, J.A.; Filla, M.S.; Shah, N.S.; Sun, Y.Y.; Keller, K.E.; Peters, D.M. Age-Related Dysregulation of α5β1 and αvβ3 Integrin Activity Alters Contractile Properties of Trabecular Meshwork Cells. *Investig. Ophthalmol. Vis. Sci.* **2025**, *66*, 31. [CrossRef] [PubMed]
3. Adelpour, M.; Moghimi, S.; Nishida, T.; Meller, L.; Du, K.H.; Kamalipour, A.; Tansuebchueasai, N.; Mahmoudinezhad, G.; Zangwill, L.M.; Weinreb, R.N. Short-Term Rates of Visual Field Change Predict Glaucoma Progression. *Ophthalmol. Glaucoma* **2025**, *ahead of print*. [CrossRef] [PubMed]
4. Tonti, E.; Tonti, S.; Mancini, F.; Bonini, C.; Spadea, L.; D'Esposito, F.; Gagliano, C.; Musa, M.; Zeppieri, M. Artificial Intelligence and Advanced Technology in Glaucoma: A Review. *J. Pers. Med.* **2024**, *14*, 1062. [CrossRef] [PubMed]
5. Wu, J.H.; Lin, S.; Moghimi, S. Application of artificial intelligence in glaucoma care: An updated review. *Taiwan J. Ophthalmol.* **2024**, *14*, 340–351. [CrossRef]
6. Ravindranath, R.; Stein, J.D.; Hernandez-Boussard, T.; Fisher, A.C.; Wang, S.Y.; SOURCE Consortium. The Impact of Race, Ethnicity, and Sex on Fairness in Artificial Intelligence for Glaucoma Prediction Models. *Ophthalmol. Sci.* **2024**, *5*, 100596. [CrossRef] [PubMed]
7. Shan, S.; Wu, J.; Cao, J.; Feng, Y.; Zhou, J.; Luo, Z.; Song, P.; Rudan, I.; Global Health Epidemiology Research Group (GHERG). Global incidence and risk factors for glaucoma: A systematic review and meta-analysis of prospective studies. *J. Glob. Health* **2024**, *14*, 04252. [CrossRef] [PubMed]
8. Mastronikolis, S.; Pagkalou, M.; Baroutas, G.; Kyriakopoulou, K.; Makri, O.E.; Georgakopoulos, C.D. Pseudoexfoliation syndrome: The critical role of the extracellular matrix in pathogenesis and treatment. *IUBMB Life* **2022**, *74*, 995–1002. [CrossRef] [PubMed]
9. Ghaffari Sharaf, M.; Damji, K.F.; Unsworth, L.D. Recent advances in risk factors associated with ocular exfoliation syndrome. *Acta Ophthalmol.* **2020**, *98*, 113–120. [CrossRef] [PubMed]
10. Qiao, C.Y.; Wang, S.Y. Standardizing the diagnosis and treatment of ocular hypertension based on evidence-based medicine. *Chin. Med. J.* **2024**, *104*, 2195–2199.
11. Svedberg, E.; Ekström, C. Distribution of cup-disc ratio in a Swedish population. *Upsala J. Med. Sci.* **2023**, *128*, 10-48101. [CrossRef] [PubMed]
12. Ekström, C.; Carlsson, C. Incidence of blindness in open-angle glaucoma in Sweden: A long-term follow-up study. *Upsala J. Med. Sci.* **2024**, *129*, 10-48101. [CrossRef] [PubMed]
13. Wang, Y.X.; Yang, H.; Wei, C.C.; Xu, L.; Wei, W.B.; Jonas, J.B. High myopia as risk factor for the 10-year incidence of open-angle glaucoma in the Beijing Eye Study. *Br. J. Ophthalmol.* **2023**, *107*, 935–940. [CrossRef] [PubMed]
14. Spaeth, G.L. European Glaucoma Society Terminology and Guidelines for Glaucoma, 5th edition. *Br. J. Ophthalmol.* **2021**, *105*, 1–169. [CrossRef]
15. Wolfram Research, Inc. *Mathematica*, version 13.3; Wolfram Research: Champaign, IL, USA, 2023.
16. Franc, V.; Prusa, D.; Voracek, V. Optimal strategies for reject option classifiers. *J. Mach. Learn. Res.* **2023**, *24*, 1–49.
17. Cloud Software Group, Inc. *Spotfire*, version 14, Data Science Workbench. Cloud Software Group: Fort Lauderdale, FL, USA, 2023. Available online: https://www.spotfire.com/ (accessed on 28 October 2024).

18. Lan, C.H.; Chiu, T.H.; Yen, W.T.; Lu, D.W. Artificial Intelligence in Glaucoma: Advances in Diagnosis, Progression Forecasting, and Surgical Outcome Prediction. *Int. J. Mol. Sci.* **2025**, *26*, 4473. [CrossRef]
19. Carlà, M.M.; Gambini, G.; Giannuzzi, F.; Boselli, F.; De Luca, L.; Rizzo, S. Testing the Reliability of ChatGPT Assistance for Surgical Choices in Challenging Glaucoma Cases. *J. Pers. Med.* **2025**, *15*, 97. [CrossRef]
20. Wąż, P.; Zorena, K.; Murawska, A.; Bielińska-Wąż, D. Classification Maps: A New Mathematical Tool Supporting the Diagnosis of Age-Related Macular Degeneration. *J. Pers. Med.* **2023**, *13*, 1074. [CrossRef] [PubMed]
21. Demir, F.; Burak, T. An Effective and Robust Approach Based on R-CNN+LSTM Model and NCAR Feature Selection for Ophthalmological Disease Detection from Fundus Images. *J. Pers. Med.* **2021**, *11*, 1276. [CrossRef] [PubMed]
22. Govindaiah, A.; Baten, A.; Smith, R.T.; Balasubramanian, S.; Bhuiyan, A. Optimized Prediction Models from Fundus Imaging and Genetics for Late Age-Related Macular Degeneration. *J. Pers. Med.* **2021**, *11*, 1127. [CrossRef] [PubMed]

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Success of Strabismus Surgery in Intermittent Exotropia

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Abstract: Introduction: Intermittent exotropia (IXT) is the most common form of childhood divergent strabismus. Surgery remains the primary approach to control ocular deviation and preserve binocular function. Although previous studies report a success rate of approximately 75%, factors influencing surgical outcomes remain insufficiently explored. This study evaluates the effectiveness of strabismus surgery in children with IXT and identifies predictors of postoperative alignment stability. **Methods**: This retrospective study included 258 children with basic-type IXT or divergence excess who underwent bilateral lateral rectus recession. Clinical records and surgical data were analyzed to determine the overall success rate and identify associated predictive factors. **Results**: The sample included 166 females (64.3%) and 92 males (35.7%), with a mean age of 11.19 $\pm$ 3.73 years. Surgical success was achieved in 238 patients (92.2%). Success rates were similar across sexes (92.8% in females vs. 91.3% in males). No significant associations were found between surgical success and sex, age, preoperative occlusion therapy, binocular function, or IXT subtype. However, patients with moderate preoperative deviations had higher success rates. A statistically significant difference was observed in the preoperative deviation angle between successful (31 $\pm$ 7.08Δ) and unsuccessful (42 $\pm$ 7.27Δ) cases. A lower AC/A ratio was also associated with better outcomes, although it was not the main predictor. **Discussion**: The high success rate (92.2%) suggests a limited impact of demographic or preoperative variables. The preoperative deviation angle emerged as the strongest predictor of success, with smaller angles correlating with more favorable surgical outcomes. These findings underscore the importance of accurate preoperative assessment in surgical planning for IXT.

Keywords: intermittent exotropia; surgery; surgical success; recurrence

1. Introduction

Intermittent exotropia (IXT) is the most common type of divergent strabismus in children. It is defined as a deviation that is present intermittently and alternates with periods of orthotropia [1–3]. The deviation usually appears during early childhood (between 1 and 5 years of age) and typically becomes more frequent over time. It often manifests during distance fixation or in situations of fatigue, distraction, or illness. As the child grows, the deviation may also appear at near fixation and can eventually progress to constant

exotropia (XT) [1]. The development of amblyopia is rare and usually occurs only when the condition becomes constant at an early age or when it coexists with other amblyogenic factors, such as uncorrected refractive errors [1].

Typically, during periods of ocular alignment, individuals exhibit binocular vision and stereopsis. When the deviation is present, anomalous retinal correspondence (ARC) may occur. Depending on the patient's age and the maturity of the visual system, suppression or diplopia may develop [1–5].

According to Duane's classification, IXT can be divided into four types: divergence excess, basic type, convergence insufficiency, and pseudo-divergence excess [1,5].

There is currently no universally accepted first-line treatment for IXT [1]. Treatment options include medical approaches, such as occlusion therapy and the use of minus lenses to stimulate convergence, and orthoptic therapy, consisting of fusion exercises, which remain controversial due to ongoing debate regarding their effectiveness and their potential negative impact on surgical outcomes. Orthoptic therapy may be useful only in cases of convergence insufficiency, as it can promote fixation and improve fusion. Therefore, surgical intervention is the mainstay of treatment for most IXT cases. Surgery typically yields satisfactory results, although undercorrection or overcorrection may occur in the immediate postoperative period. Over time, XT may recur, and additional surgery may be required [1,4–7]. Surgery for IXT has reported success rates of 70–80%, but recurrence can occur in up to 50% of cases within three years. Several prognostic factors strongly influence outcomes [8–14]. A smaller preoperative deviation angle is associated with better surgical success and a reduced need for reoperation [5,8,10,15]. Younger age at the time of surgery, particularly between 3 and <5 years, is also linked to higher success rates [8,14]. The type of exotropia is another important factor: the pseudo-divergence excess subtype is associated with better alignment and lower recurrence [14–16]. Postoperative alignment during the first week is a strong predictor of long-term success, especially in adults. In cases with large deviation angles (>60 PD), the likelihood of achieving orthophoria is high, although binocular success may be limited [5,9]. A family history of strabismus surgery does not appear to significantly influence surgical outcomes [4].

This study aims to evaluate the effectiveness of bilateral lateral rectus recession surgery in children with intermittent exotropia (IXT), with a particular focus on identifying predictive factors for long-term alignment stability. In contrast to previous studies, this research provides a comprehensive analysis of a large pediatric cohort in Portugal, applying a personalized medicine approach to stratify surgical outcomes based on clinical profiles such as preoperative deviation angle and AC/A ratio.

2. Methodology

This was a retrospective study [17].of patients treated at CUF Cascais Hospital, involving a cohort of children who underwent strabismus surgery for intermittent exotropia. The procedure performed was classic bilateral lateral rectus recession under general anesthesia. All surgeries were conducted by the same surgeon, and the surgical dose was determined based on the distance deviation angle using Park's formula (Table 1).

Inclusion criteria: Children aged 4 to 18 years with a confirmed diagnosis of intermittent exotropia who underwent surgical correction. Only patients with complete medical records—including preoperative data, surgical reports, and postoperative follow-up—were included. All participants had normal visual acuity (i.e., no amblyopia) and no associated ocular or systemic conditions that could influence the evaluation of surgical outcomes.

Table 1. Parks' formula for exotropia surgery.

Exotropia Angle in PD	Amount of Recession/Resection in Mm	
Two muscles	BLRc	LRc + MRs (R&R)
15	4.0	4.0 + 3.0
20	5.0	5.0 + 4.0
25	6.0	6.0 + 4.0
30	7.0	7.0 + 5.5
35	7.5	7.5 + 5.5
40	8.0	8.0 + 5.5
45	8.5	8.5 + 6.0
50	9.0	9.0 + 6.5
60	10	9.0 + 7.0

Legend: PD, prismatic diopters. BLRc, bilateral lateral rectus recession. LRc, lateral rectus recession. MRs: medial rectus resection.

Exclusion criteria: Patients were excluded if they presented other ocular or systemic pathologies, unilateral or bilateral amblyopia, chromosomal abnormalities or systemic diseases (e.g., congenital anomalies or neurological disorders), paralytic or restrictive exotropia, dissociated vertical deviation or alphabet patterns, constant exotropia, or other types of divergent strabismus such as consecutive or sensory exotropia.

The following parameters were documented and analyzed: sex, age, age at surgery, refractive error (spherical equivalent), distance deviation in prism diopters (PD) before surgery, preoperative sensory status, type of surgical procedure performed, postoperative deviation in PD at six months, and sensory function at six months. A six-month follow-up was chosen because ocular alignment is generally more stable after this period, allowing for a more accurate assessment of residual deviation. This timeframe also permits evaluation of sensory adaptation (e.g., binocular fusion), identification of relapses or overcorrections, and assessment of symptoms and quality of life. Moreover, it is a commonly used interval in scientific literature, facilitating comparison with other studies.

In all patients, deviations were measured using the alternate cover test with prisms for both near (33 cm) and distance (6 m) fixation. To differentiate between true divergence excess and pseudo-divergence excess, measurements were repeated after occlusion (1 h) of the non-dominant eye. The AC/A ratio was calculated using the gradient method, with the addition of -2.00 D lenses.

Sensory status was evaluated using fusion (Worth 4-dot test) and stereopsis (Wirt test for near, Vectograph for distance).

Definition of surgical success: Residual ocular deviation $\leq$10 PD at distance fixation six months after surgery (whether eso- or exotropic), with the presence of sensory fusion and stereopsis of 80 arcseconds or better, as assessed by either near (Wirt) or distance (Vectograph) stereotests. This combined motor and sensory criterion aligns with prior literature and enables a comprehensive evaluation of functional surgical outcomes.

Statistical analysis [18,19]: Data were entered into a custom SPSS® version 29 database and analyzed using univariate, bivariate, and multivariate statistical methods. Variables were classified according to their characteristics and categorized accordingly.

Qualitative sociodemographic and clinical variables were analyzed using descriptive statistics, and quantitative variables were summarized using measures of central tendency and dispersion. Logistic regression was used to identify factors associated with stable post-operative alignment, including both crude and adjusted models. Variables included in the

model were selected based on clinical relevance and statistical significance in bivariate analysis, and multicollinearity was assessed to ensure model robustness. A significance level of 5% was adopted, and *p*-values below this threshold were considered statistically significant.

All stages of the research complied with ethical and regulatory standards for Good Practice in Health Research and were approved by the Ethics Committee of the CUF Group—Health Services (approval number: 2024/488).

3. Results

The sample included 258 participants: 166 females (64.3%) and 92 males (35.7%). The participants' ages ranged from 5 to 18 years, with a mean age of 11.19 ± 3.73 years.

The mean age at the time of surgery was 8.75 ± 12.19 years, with a minimum of 4 years and a maximum of 17 years.

Out of the total sample, 238 patients (92.2%) achieved surgical success, defined as a residual eso- or exodeviation ≤10 PD and stereopsis better than 80 arcseconds. The remaining 20 patients (7.8%) did not meet the criteria for success. In the success group, the mean refractive error (spherical equivalent) was −0.63 ± 1.424 D, while in the no-success group, it was −0.63 ± 1.134 D.

Ophthalmologic characteristics: Table 2 shows the associations between each of the characteristics studied in each of the groups (success vs. non-success).

Table 2. Association between exposures and outcome.

Exposure	Group: Success	Group: No Success	OR	*p*-Value	95% Confidence Interval
Gender					
Female (reference)	154 (92.8%)	12 (7.2%)			
Male	84 (91.3%)	8 (8.7%)	0.818	0.673	0.322; 2.080
Age	11.22 ± 3.589	12.20 ± 5.414	1.031	0.627	0.911; 1.168
Surgical Age	8.76 ± 12.617	9.80 ± 5.519	1.001	0.954	0.962; 1.042
Prior occlusive treatment					
Yes	196 (91.2%)	19 (8.8%)			
No (reference)	42 (97.7%)	1 (2.3%)	0.246	0.177	0.032; 1.886
XTI type					
DET	51 (89.5%)	6 (10.5%)			
BT (reference)	187 (93%)	14(7%)	0.636	0.378	0.233; 1.739
Moderate preoperative deviation					
Yes	76 (100%)	0(0%)			
No (reference)	162 (89%)	20 (11%)	9.259 *	0.01	1.648; 196.3
Magnitude of preoperative deviation	31 ± 7.080	42 ± 7.270	0.837	<0.001	0.777; 0.901
AC/A ratio	7.09 ± 3.735	11.27 ± 9.035	0.920	0.013	0.861; 0.983
Binocular Preoperative					
Yes	233 (92.5%)	19 (7.5%)			
No (reference)	5 (83.3%)	1 (16.7%)	2.453	0.424	0.272; 22.077
Surgical Dose (mm)	13.65 ± 2.5	15.33 ± 3.395	0.817	0.025	0.685; 0.975

* For this calculation, the authors do a direct substitution of 0 by 1 OR (odds ratio).

- Preoperative occlusive treatment: 196 patients (91.2%) underwent occlusion therapy as an anti-suppression exercise.
- Binocular function before surgery: 233 patients (92.5%) had binocular function prior to surgery. The odds ratio (OR) for surgical success in patients with binocular vision was 2.453 ($p = 0.424$).
- IXT type: 187 patients (93%) had basic-type intermittent exotropia, and 51 patients (89.5%) had divergence excess type. The OR for surgical success in patients with basic-type IXT was 0.636 ($p = 0.378$).
- Preoperative deviation angle: The mean deviation at distance was 31 $\pm$ 7.08Δ in the success group and 42 $\pm$ 7.27Δ in the no-success group. Patients with moderate deviation had a significantly higher chance of surgical success.
- AC/A ratio: The mean AC/A ratio was 7.09 $\pm$ 3.735 in the success group and 11.27 $\pm$ 9.035 in the no-success group. A statistically significant difference was found (OR = 0.920; $p = 0.013$), suggesting that a higher AC/A ratio is associated with lower surgical success.
- Total surgical dose: The mean total amount of recession (bilateral) was 13.65 $\pm$ 2.5 mm in the success group and 15.33 $\pm$ 3.395 mm in the no-success group. Although there was no statistically significant difference between larger setbacks (t = 1.891; $p = 0.078$), logistic regression showed a significant association ($p = 0.025$).
- Sex: Surgical success was achieved in 154 females (92.8%) and 84 males (91.3%). Although females tended to have a slightly higher success rate, the difference was not statistically significant (OR = 0.818; $p = 0.673$).
- Age and age at surgery: The mean age in the success group was 11.22 $\pm$ 3.589 years, compared to 12.20 $\pm$ 5.414 years in the no-success group. The mean age at surgery was 8.76 $\pm$ 12.617 years in the success group and 9.80 $\pm$ 5.519 years in the no-success group. There was no significant association between these variables and surgical success, although younger patients tended to have better outcomes (OR = 1.031; $p = 0.627$ for age; OR = 1.001; $p = 0.954$ for surgical age).

By further analyzing associations and interactions between variables using binary logistic regression, it was found that the preoperative deviation angle was the only exposure significantly influencing surgical outcome.

In the optimized logistic regression model (which included variables with $p < 0.2$ and excluded those with adjusted $p > 0.05$ after testing for collinearity), the only statistically significant predictor of surgical success was the preoperative deviation angle: patients with smaller deviation angles had a significantly higher probability of successful outcomes (OR = 0.837; $p < 0.001$).

4. Discussion

The primary objective of surgical intervention in intermittent exotropia (IXT) is to improve ocular alignment while preserving or enhancing binocular function. Therefore, surgical decisions are generally based on the clinical characteristics and functional impact of the strabismus. Large-angle exodeviations associated with reduced fusional amplitudes and a tendency toward loss of binocularity are typical indications for surgery.

4.1. Surgical Outcomes and Success Rates:

This study included 258 patients—166 females (64.3%) and 92 males (35.7%)—with ages ranging from 5 to 18 years (mean: 11.19 $\pm$ 3.73 years). The mean age at the time of surgery was 8.75 $\pm$ 12.19 years, with a minimum of 4 years and a maximum of 17 years. Surgical success was achieved in 238 patients (92.2%), while 20 patients (7.8%) did not achieve the defined criteria for success.

The overall surgical success rate in this study—exceeding 90%—is higher than what has been reported in several previous studies. For example, Donahue et al. reported a success rate of approximately 70% in a cohort of 197 patients in Florida [20]. This study represents the first publication from Portugal addressing bilateral surgery for IXT. Although a previous study with a small sample size reported a 100% success rate [4], our findings are based on a larger and more representative cohort. Reported success rates in the literature vary depending on the follow-up period. Kopmann et al. reported a success rate of 83.8% on the first postoperative day [5], while Thorisdottir et al. reported an 80% success rate at two months postoperatively [21]. In this context, our success rate at six months supports the high efficacy of bilateral lateral rectus muscle recession for IXT.

4.2. Factors Influencing Surgical Outcomes:

- Preoperative deviation angle: Statistical analysis showed that a smaller preoperative deviation angle was associated with higher surgical success. The mean angle in the success group was 31 $\pm$ 7.08Δ, compared to 42 $\pm$ 7.27Δ in the no-success group. Patients with moderate deviations were significantly more likely to achieve success (OR = 9.259; $p = 0.01$). Quantitative analysis further confirmed the protective role of a smaller angle (OR = 0.837; $p < 0.001$), indicating that each additional prism diopter increased the risk of failure by approximately 16%. These results are consistent with previous findings, including those of Kopmann et al. (2024) [5], Tibrewal (2017) [22], and Thorisdottir (2022) [21], all of which suggest that patients with smaller preoperative angles have better outcomes.
- Age at surgery: The mean age in the success group was 11.22 $\pm$ 3.59 years, compared to 12.20 $\pm$ 5.41 years in the no-success group. The mean surgical age was 8.76 $\pm$ 12.62 years in the success group and 9.80 $\pm$ 5.52 years in the no-success group. Although no statistically significant association was found ($p = 0.627$ for age; $p = 0.954$ for surgical age), there was a tendency toward better results in younger patients. This is in agreement with Issaho et al. (2017) [11], although some authors, such as Jiménez-Romo (2023), argue that age is not a significant predictor of surgical success [23].
- Binocular function: Preoperative binocular function was present in 233 patients (92.5%). Although a tendency toward better outcomes in these patients was observed (OR = 2.453; $p = 0.424$), the association was not statistically significant. This suggests that surgery may either preserve or restore binocular function, depending on the preoperative status.
- AC/A ratio: The mean AC/A ratio was 7.09 $\pm$ 3.73 in the success group and 11.27 $\pm$ 9.04 in the no-success group. A higher AC/A ratio was significantly associated with lower surgical success (OR = 0.920; $p = 0.013$), indicating that patients with greater accommodative convergence relative to accommodation are less likely to benefit from surgery [24].
- Gender: Surgical success was achieved in 154 female patients (92.8%) and 84 male patients (91.3%). Although there was a slight trend toward higher success in females (OR = 0.818; $p = 0.673$), the difference was not statistically significant and may be due to chance.
- Preoperative occlusion therapy: Occlusion therapy was performed in 196 patients (91.2%). While it appeared to be a potential protective factor (OR = 0.246; $p = 0.177$), the association was not statistically significant. This finding is in line with the anterior works [14], who suggested that children undergoing occlusion therapy before surgery may have better outcomes.
- Surgical dose: The mean bilateral recession was 13.65 $\pm$ 2.5 mm in the success group and 15.33 $\pm$ 3.39 mm in the no-success group. Although the direct comparison did not

reach statistical significance ($p = 0.078$), logistic regression revealed a significant negative association between larger recession amounts and surgical success (OR = 0.817; $p = 0.025$), indicating that higher surgical doses may be associated with a lower likelihood of favorable outcomes.

4.3. Study Limitations:

This study has several limitations, primarily related to its retrospective design. Patients were not randomly assigned to treatment protocols; instead, the surgical approach was based on the clinical judgment and experience of the operating surgeon. This may introduce selection bias and limit the generalizability of our findings. Nevertheless, the large sample size and comprehensive analysis strengthen the conclusions and provide meaningful insights into the factors influencing surgical success in IXT.

5. Conclusions

Children diagnosed with the basic type of intermittent exotropia tended to achieve more favorable surgical outcomes. After adjusting for potential confounding variables, a statistically significant association was found between smaller preoperative deviation angles and a greater likelihood of surgical success.

Although younger age at the time of surgery and the presence of preoperative binocular function did not reach statistical significance, these factors may still hold clinical relevance and should be considered during surgical planning and decision-making.

Author Contributions: P.L., P.V.d.A. and J.P.C. designed the study. P.L. collected the data under the supervision of P.V.d.A. and J.P.C. P.L. analyzed the data with statistical guidance from P.V.d.A. and scientific input from J.P.C. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: The authors have given their consent for the publication of this manuscript. As this was a retrospective study with a positive opinion from the ethics committee, the requirement for informed consent was waived.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

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References

1. Tejedor, J.Y.C. *Strabismus and Amblyopia*, 1st ed.; McGraw-Hill Interamericana de España S.L.: Madrid, Spain, 2022.
2. Jeanrot, N.; Ducret, V. *Manuel de Strabologie: Aspects Cliniques et Thérapeutiques*; Elsevier-Masson: Issy-les-Moulineaux, France, 2018.
3. von Noorden, G.K.; Campos, E. *Binocular Vision and Ocular Motility*, 6th ed.; Mosby: Maryland Heights, MO, USA, 2022.
4. Cristóvão, D.; Seldon, R.; De, M.; Vieira, L. Exotropia intermitente: From surgical success to long-term need for reoperation. *Oftalmologia* **2013**, *37*, 259. (In Portuguese)
5. Kopmann, S.; Grenzebach, U.; Ehrt, O.; Biermann, J. Effectiveness of strabismus surgery in intermittent exotropia and factors influencing outcome. *J. Clin. Med.* **2024**, *13*, 1031. [CrossRef] [PubMed]
6. Anand, K.; Krishnan, A.; Kumar, P.; Bharadwaj, A.; Dutta, P.; Rastogi, A.; Hariani, A. Comparative Study of Bilateral Versus Unilateral Strabismus Surgery in the Management of Lateral Incomitance in Intermittent Exotropia. *J. Pediatr. Ophthalmol. Strabismus* **2023**, *60*, 277–281. [CrossRef] [PubMed]
7. Yildirim, C.; Mutlu, F.M.; Chen, Y.; Altinsoy, H.I. Assessment of central and peripheral fusion and near and distance stereoacuity in intermittent exotropic patients before and after strabismus surgery. *Am. J. Ophthalmol.* **1999**, *128*, 222–230. [CrossRef] [PubMed]

8. Rajavi, Z.; Hafezian, S.F.; Yaseri, M.; Sheibani, K. Early postoperative alignment as a predictor of 6-month alignment after intermittent exotropia surgery. *J. Pediatr. Ophthalmol. Strabismus* **2014**, *51*, 274–282. [CrossRef] [PubMed]
9. Huh, J.; Ha, S.G.; Kim, S.H. Recovery From Suppression with Successful Motor Alignment After Surgery for Intermittent Exotropia. *J. Pediatr. Ophthalmol. Strabismus* **2020**, *57*, 21–26. [CrossRef] [PubMed]
10. Maruo, T.; Kubota, N.; Sakaue, T.; Usui, C. Intermittent exotropia surgery in children: Long term outcome regarding changes in binocular alignment. A study of 666 cases. *Binocul. Vis. Strabismus Q.* **2001**, *16*, 265–270. [PubMed]
11. Issaho, D.C.; Wang, S.X.; Weakley, D.R. Intermittent exotropia surgery: Results in different age groups. *Arq. Bras. Oftalmol.* **2017**, *80*, 355–358. [CrossRef] [PubMed]
12. Xu, M.; Zheng, F.; Peng, Y.; Wang, C.; Lou, J.; Yu, H.; Wang, Y.; Yu, X. Effects of orthoptic therapy in children with intermittent exotropia after surgery: Study protocol for a randomized controlled trial. *Trials* **2022**, *23*, 1123. [CrossRef] [PubMed]
13. Farid, M.F.; Abdelbaset, E.A. Surgical outcomes of three different surgical techniques for treatment of convergence insufficiency intermittent exotropia. *Eye* **2018**, *32*, 693–700. [CrossRef] [PubMed]
14. Bae, G.H.; Bae, S.H.; Choi, D.G. Surgical outcomes of intermittent exotropia according to exotropia type based on distance/near differences. *PLoS ONE* **2019**, *14*, e0214478. [CrossRef] [PubMed]
15. Hong, S.; Ha, S.; Seo, Y.; Kim, S. Analysis of Postoperative Outcomes in Patients with Intermittent Exotropia According to Preoperative Change of Angle of Deviation. *Korean J. Ophthalmol. KJO* **2021**, *35*, 130–135. [CrossRef] [PubMed]
16. Lavrich, J.B. Intermittent exotropia: Continued controversies and current management. *Curr. Opin. Ophthalmol.* **2015**, *26*, 375–381. [CrossRef] [PubMed]
17. Fortin, M.F. *Fundamentos E Etapas no Processo de Investigação*; Lusodidacta: Sintra, Portugal, 2009.
18. Oliveira, A. *Bioestatística Descodificada*, 2nd ed.; LIDEL—Edições Técnicas, Lda.: Lisboa, Portugal, 2014; Volume 1.
19. Vicente, V.; Aguiar, P. *Practical Epidemiology with GLM*; Editora d'Ideias: Coimbra, Portugal, 2023.
20. Donahue, S.P.; Chandler, D.L.; Wu, R.; Marsh, J.D.; Law, C.; Areaux, R.G., Jr.; Ghasia, F.F.; Li, Z.; Kraker, R.T.; Cotter, S.A.; et al. Eight-Year Outcomes of Bilateral Lateral Rectus Recessions versus Unilateral Recession-Resection in Childhood Basic-Type Intermittent Exotropia. *Ophthalmology* **2024**, *131*, 98–106. [CrossRef] [PubMed]
21. Thorisdottir, R.; Malmsjö, M.; Tenland, K.; Blohmé, J.; Hesgaard, H. The success of unilateral surgery for constant and intermittent exotropia and factors affecting it in a large Scandinavian case series. *J. Pediatr. Ophthalmol. Strabismus* **2021**, *58*, 34–41. [CrossRef] [PubMed]
22. Tibrewal, S.; Singh, N.; Bhuiyan, M.; Ganesh, S. Factors affecting residual exotropia after two muscle surgery for intermittent exotropia. *Int. J. Ophthalmol.* **2017**, *10*, 1120–1125. [CrossRef] [PubMed]
23. Jiménez-Romo, C.A.; Rangel-Padilla, A.; Páez-Garza, J.H. Timely Surgery in Intermittent Exotropia. *J. Binocul. Vis. Ocul. Motil.* **2023**, *73*, 21–27. [CrossRef] [PubMed]
24. Brodsky, M.C.; Horwood, A.M.; Riddell, P.M. Intermittent exotropia: Are we underminusing by not overminusing? *J. AAPOS* **2015**, *19*, 397–398. [CrossRef] [PubMed]

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Effect of Prior Laser-Assisted In Situ Keratomileusis on the Calibration Accuracy of Extended Depth of Focus Intraocular Lenses: A Direct Comparative Study

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Abstract: Background: Personalized precision medicine has become a prevailing trend and applies to the selection of intraocular lenses (IOLs) for cataract surgery based on the unique corneal morphology of each person. The choice of presbyopia-correcting IOLs for post-laser-assisted in situ keratomileusis (LASIK) cataract surgery is a significant concern. However, few direct comparison studies exist between eyes with and without LASIK history. We analyzed the performance of extended depth of focus (EDOF) IOL implantation in these two groups. **Methods:** In this retrospective single-center study, we included patients with or without previous LASIK who underwent cataract surgery and EDOF Symfony IOL implantation, with ≥1 follow up. All patients underwent optical biometry using the IOLMaster. IOL power was calculated using the Sanders Retzslaff Kraff/theoretical and Haigis-L formulas for patients without and with LASIK, respectively. Uncorrected distance visual acuity (UDVA), uncorrected near visual acuity (UNVA), refraction, and corneal tomography were recorded. The prediction error was the absolute difference between the postoperative sphere and target refraction. The right eyes of patients who met the inclusion criteria were selected for analysis. **Results:** Among the 321 recruited eyes, 18 underwent previous LASIK. After 1:3 age/sex matching, 17 LASIK and 49 non-LASIK eyes from 66 patients were analyzed. No significant preoperative differences existed in target refraction, spherical equivalent, or best-corrected visual acuity. All surgical procedures were uneventful. LASIK exhibited non-inferiority to non-LASIK for predictive refraction error and UNVA. An age/sex-matched regression analysis indicated no UDVA superiority between the two groups. **Conclusions:** Previous LASIK may have no discernible effect on the visual performance of presbyopia-correcting EDOF IOLs with respect to the absolute refractive error, UNVA, and UDVA. Longer follow-up and larger-scale studies are required to further validate these results.

Keywords: laser in situ keratomileusis; cataract; extended depth of focus; absolute prediction error

1. Introduction

Laser-assisted in situ keratomileusis (LASIK) is a widely utilized refractive surgery for correcting myopia and astigmatism by reshaping the corneal stroma with an excimer

laser. This reshaping allows the focal point of incoming light to be realigned onto the retina, thereby improving visual acuity [1]. While LASIK is highly effective in reducing the dependence on corrective lenses, it can also alter corneal biomechanics and induce higher-order aberrations, such as coma and spherical aberration. These optical changes may influence visual quality, although their impact on contrast sensitivity remains debated [2].

With the aging of LASIK-treated individuals, an increasing number of these patients are now undergoing cataract surgery. Nowadays, personalized precision medicine has become a prevailing trend. The same applies to the selection of intraocular lenses (IOLs) for cataract surgery. We should choose the most suitable IOL based on the unique corneal morphology of each individual. The selection of an appropriate intraocular lens (IOL) for post-LASIK eyes presents unique challenges due to the altered corneal biomechanics and shape, which affects the accuracy of IOL power calculations. Traditional IOL calculation formulas often yield suboptimal refractive outcomes in post-LASIK eyes, necessitating specialized formulas or newer technologies to enhance predictability. Among various IOL options, multifocal and extended depth of focus (EDOF) IOLs have been proposed as potential solutions for presbyopia correction in these patients. However, concerns remain regarding their performance in eyes with previous LASIK, particularly in terms of visual quality, dysphotopsia, and contrast sensitivity.

Although some studies have suggested that multifocal IOLs can be successfully implanted in post-LASIK eyes under specific conditions—such as the presence of regular corneal astigmatism and the use of optimized IOL power calculation methods [3,4]—comparative data between post-LASIK and non-LASIK eyes remain limited. In particular, direct assessments of EDOF IOL performance in post-LASIK patients are scarce. Given the growing number of patients seeking spectacle independence after cataract surgery, a clearer understanding of how EDOF IOLs function in post-LASIK eyes is essential for optimizing surgical outcomes and patient satisfaction.

Against this background, this study aims to provide a comparative analysis of EDOF IOL implantation outcomes in patients with and without a history of LASIK. By evaluating the postoperative visual performance, we seek to determine whether EDOF IOLs are a viable option for individuals who have previously undergone LASIK.

2. Materials and Methods

2.1. Study Design and Ethical Approval

Patients who visited the Taipei Nobel Eye Clinic from 2022 to 2023 were consecutively enrolled. This study was approved by the Ethics Committee of the National Changhua University of Education (Changhua, Taiwan) and registered on ClinicalTrials.gov (identifier NCT06165796).

2.2. Patients

The inclusion criteria comprised the following: (1) the presence of cataracts in both eyes, (2) age between 50 and 80 years, and (3) a corrected distance visual acuity (CDVA) under 20/40 in both eyes. Phacoemulsification cataract surgery was performed in all patients with or without previous myopic LASIK surgery. The exclusion criteria included the following: (1) complicated cataracts, (2) corneal opacities or irregularities, (3) corneal astigmatism > 1.50 diopters, (4) dry eye (Schirmer's test I $\leq$ 5 mm), (5) amblyopia, (6) anisometropia, (7) surgical complications such as posterior capsular bag rupture or vitreous loss, (8) IOL tilt or decentration, (9) coexisting ocular pathologies, (10) glaucoma, (11) non-dilating pupil, (12) history of intraocular surgery or retinopathy, (14) optic nerve or macular diseases, and (15) refusal or inability to maintain follow-up. Patients with optic zone decentration or irregular astigmatism in the LASIK group were excluded. Among

patients who visited the Taipei Nobel Eye Clinic between 2022 and 2023, 331 eyes of 186 patients were recruited for this study.

2.3. Surgery

Clear corneal phacoemulsification and IOL implantation were performed by the same surgeon (Chao-Kai Chang). The surgical procedure involved the application of topical anesthesia, a three-step clear corneal incision (2.25 mm) at 180° (temporal in both eyes), a 5.0 mm continuous curvilinear capsulorhexis, phacoemulsification using the stop-and-chop technique, IOL implantation with an injector, IOL centration, and sutureless incisions. We exclusively used EDOF Symfony IOLs (Johnson & Johnson, Santa Ana, CA, USA).

2.4. Ophthalmic Examinations

The patients underwent comprehensive preoperative examinations and were subsequently assessed at 1 d, 1 week, and 1 month postoperatively. Each ophthalmological examination included tests for uncorrected distance visual acuity (UDVA) and CDVA at far and near distances, manifest refraction, biomicroscopy, and applanation tonometry. Corneal curvature values were exported from a Pentacam machine (Pentacam HR; Oculus GmbH, Wetzlar, Germany) using a Scheimpflug keratometry system. Fundus examinations were performed preoperatively. Preoperatively, all patients underwent optical biometry using the IOLMaster (IOLMaster 500, Carl Zeiss Meditec AG, Jena, Germany). The IOL power was calculated using the Sanders Retzlaff Kraff/theoretical (SRK/T) formula for patients without LASIK and the Haigis-L formula for patients with LASIK. The postoperative refraction target was emmetropia. This means that based on the post-IOL implantation refraction degree predicted by the IOLMaster, we chose an IOL as close to 0 degrees as possible for the IOL implantation, and that degree was defined as target refraction. Postoperatively, the IOL concentration was additionally evaluated using retro-illumination.

2.5. Statistical Analysis

The main post-surgery measurements used to compare the performance between patients with and without LASIK included the following: (i) ophthalmic examinations assessing UDVA, CDVA, and uncorrected near visual acuity (UNVA) at 40 cm and (ii) prediction error, defined as the absolute difference between the postoperative manifest sphere and target refraction. By using a model of generalized estimating equations (GEE), the regression analysis was adjusted for the following covariates: age, sex, axial length, anterior chamber depth (ACD), corneal curvature (K), IOL power, and preoperative sphere.

Non-inferiority tests [5] were conducted to assess whether the LASIK group's performance was not inferior to that of the non-LASIK group with respect to three post-cataract surgery outcomes: absolute predictive error, UDVA, and UNVA. The null hypothesis posits that the difference in the group mean is not less than the inferiority margin, in contrast to the alternative hypothesis that the difference in the group mean is less than the inferiority margin. To assess the non-inferiority of the LASIK group compared with the non-LASIK group, a 95% confidence interval of the difference in the means of target refraction between the groups was constructed [5].

If the upper limit of the interval does not exceed the predefined non-inferiority margin of 0.5 diopters (D) in the prediction error, non-inferiority is confirmed. Similarly, for UNVA and UDVA, the predefined non-inferiority margins are both a 0.1 logarithm of the minimum angle of resolution (logMAR) [6,7].

3. Results

Among the 321 recruited eyes, 18 had undergone previous LASIK, while the remaining 303 had not. After 1:3 matching for age and sex, the analysis included a final sample of

17 LASIK eyes and 49 non-LASIK eyes from 66 patients. All the 17 LASIK eyes had received LASIK surgery at ages 20 to 50. Table 1 presents the demographic characteristics of the patients and preoperative visual acuity data. No significant preoperative differences were observed in the target refraction, spherical equivalent, or best-corrected visual acuity. The LASIK eyes exhibited longer axial lengths (27.57 ± 1.43 mm) compared with the non-LASIK eyes (25.63 ± 1.80 mm), and the ACD was also deeper in LASIK eyes (3.57 ± 0.36 mm) than in non-LASIK eyes (3.34 ± 0.37 mm). A corneal topography revealed flatter K1 and K2 values (38.07 ± 2.18 and 38.82 ± 2.17, respectively) in the LASIK eyes in comparison with the non-LASIK eyes (43.13 ± 1.55 and 44.46 ± 1.81, respectively) (Table 1). All surgical procedures were performed smoothly, and all IOLs were positioned within the capsular bag.

Table 1. Demographic and clinical characteristics of patients with and without LASIK in the original and propensity-matched cohorts.

Original Data			
Previous LASIK	**LASIK (18 Eyes)**	**Non-LASIK (303 Eyes)**	
	Mean ± SD	**Mean ± SD**	***p* Value**
Preop CDVA (logMAR)	0.44 ± 0.27	0.32 ± 0.29	0.0754
Age (years)	50.78 ± 7.14	60.95 ± 8.34	<0.0001 *
Sex (M/F)	7/11	114/189	0.914
Target refraction (D)	−0.59 ± 0.38	−0.57 ± 0.30	0.8403
AXL (mm)	24.73 ± 1.66	27.60 ± 1.39	<0.0001 *
ACD (mm)	3.55 ± 0.35	3.18 ± 0.43	0.0005 *
Sphere (D)	−6.07 ± 5.35	−2.08 ± 4.76	0.0007 *
Cylinder(D)	−1.11 ± 0.8	−1.2 ± 0.90	0.6894
Flat K (D)	37.97 ± 2.15	43.44 ± 1.40	<0.0001 *
Steep K (D)	38.71 ± 2.16	44.33 ± 1.50	<0.0001 *
IOL power (D)	20.08 ± 2.85	18.39 ± 4.80	0.0289
Matched Data			
Previous LASIK	**LASIK (17 Eyes)**	**Non-LASIK (49 Eyes)**	
	Mean ± SD	**Mean ± SD**	***p* Value**
Preop CDVA (logMAR)	0.45 ± 0.27	0.34 ± 0.29	0.1879
Age (years)	51.47 ± 6.71	52.08 ± 6.26	0.7345
Sex (M/F)	7/10	21/28	0.9038
Target refraction (D)	−0.66 ± 0.26	−0.55 ± 0.29	0.2065
AXL (mm)	27.57 ± 1.43	25.63 ± 1.8	0.0001 *
ACD (mm)	3.57 ± 0.36	3.34 ± 0.37	0.0307 *
Sphere (D)	−6.28 ± 5.44	−4.82 ± 4.88	0.3063
Cylinder (D)	−1.12 ± 0.83	−1.38 ± 1.11	0.3715
Flat K (D)	38.07 ± 2.18	43.13 ± 1.55	<0.0001 *
Steep K (D)	38.82 ± 2.17	44.46 ± 1.81	<0.0001 *
IOL power (D)	20.09 ± 2.93	15.83 ± 4.83	<0.0001 *

* p value < 0.05; CDVA, corrected distance visual acuity; logMAR, logarithm of the minimum angle of resolution; AXL, axial length; ACD, anterior chamber depth; LASIK, Laser Assisted In Situ Keratomileusis; M, male; F, female; D, diopter; K, keratometry: the measurement of the corneal curvature/power; SD, standard deviation; IOL, intraocular lens.

Table 2 presents the results of the non-inferiority tests at one month post-operation. For the UNVA, the mean was 0.315 ± 0.305 logMAR in the LASIK group and 0.736 ± 1.307 logMAR in the non-LASIK group. The respective non-inferiority margins exceeded the upper bounds of their corresponding confidence intervals (non-inferiority margins: 0.1; 95% CI: −0.8143, −0.0269), confirming the non-inferiority of the LASIK group compared with the non-LASIK group. For the predictive refraction error, the mean was 0.656 ± 0.264 D

in the LASIK group and 0.551 ± 0.287 D in the non-LASIK group. The respective non-inferiority margins exceeded the upper bounds of their corresponding confidence intervals (non-inferiority margins: 0.5; 95% CI: −0.0442, 0.2541), confirming the non-inferiority of the LASIK group compared with the non-LASIK group. In contrast, for the UDVA, the mean was 0.300 ± 0.362 logMAR in the LASIK group and 0.117 ± 0.156 logMAR in the non-LASIK group. The non-inferiority margin fell within the interval, with the mean of the LASIK group being greater than that of the non-LASIK group (non-inferiority margins: 0.1; 95% CI: 0.0033, 0.3585). However, further age- and sex-matched regression analyses did not suggest that either group was significantly superior in terms of the UDVA ($p = 0.063$) (Table 3). It only revealed that the axial length has a significant influence on the UDVA ($p = 0.034$) (Table 3).

Table 2. Non-inferiority tests of predicted refractive errors and postoperative visual acuity between the LASIK and non-LASIK groups at one month post-operation.

	LASIK (SD) n = 17	Non-LASIK (SD) n = 49	Non-Inferiority Margin	95% CI	Outcome
UNVA (logMAR)	0.315 (0.305)	0.736 (1.307)	0.1	−0.8143, −0.0269	Not inferior
UDVA (logMAR)	0.300 (0.362)	0.117 (0.156)	0.1	0.0033, 0.3585	Non-LASIK is inferior to LASIK
Prediction error (D)	0.656 (0.264)	0.551 (0.287)	0.5	−0.0442, 0.2541	Not inferior

UDVA, uncorrected distance visual acuity; UNVA, uncorrected near visual acuity; logMAR, logarithm of the minimum angle of resolution; CI, confidence interval; LASIK, Laser Assisted In Situ Keratomileusis; D, diopter; SD, standard deviation.

Table 3. Age- and sex-matched regression analysis by using the model of generalized estimating equations (GEEs), showing no significant superiority between the LASIK and non-LASIK groups at one month post-operation.

Dependent Variable: PostopUDVA							
Parameter	**Level**	**Est.**	**SE**	**95% CI**		**Z**	***p* Value**
Intercept	1 (yes)	3.4494	2.2501	−0.961	7.860	1.53	0.1253
AXL		−0.1076	0.0508	−0.207	−0.008	−2.12	0.0341
Previous LASIK		0.1770	0.0952	−0.010	0.364	1.86	0.0631
ACD		0.1443	0.1278	−0.106	0.395	1.13	0.2590
Sphere		0.0140	0.0119	−0.009	0.037	1.17	0.2413
Steep K		−0.0221	0.0290	−0.079	0.035	−0.76	0.4451

UDVA, uncorrected distance visual acuity; AXL, axial length; ACD, anterior chamber depth; CI, confidence interval LASIK, Laser Assisted In Situ Keratomileusis; K, keratometry: the measurement of the corneal curvature/power; Z, z-score; SE, standard error; Est., estimates.

4. Discussion

In this retrospective single-center study, we compared the performance of EDOF IOLs after cataract surgery between eyes with and without a history of prior LASIK for myopia correction. The non-inferiority tests showed that the LASIK group was not inferior to the non-LASIK group for both the absolute predictive refractive error and the UNVA. Specifically, the upper bounds of the 95% confidence intervals for the mean differences were below the pre-defined non-inferiority margins of 0.5 D for refractive error and 0.1 logMAR for the UNVA. For the UDVA, while the non-inferiority test suggested that the LASIK group was superior, the subsequent age- and sex-matched regression analysis did not find

a statistically significant difference between the two groups after adjusting for potential confounders, such as the axial length and ACD. Our data indicate that previous LASIK did not negatively impact the visual outcomes of the EDOF IOLs in terms of predictive refractive error, UNVA, and UDVA when compared with the non-LASIK eyes in this cohort.

IOL power formulas use biometric measurements to predict the refractive outcomes of different IOLs. Measurement methods can be classified as vergence, ray tracing, artificial intelligence, or a combination of these methods [8]. These methods have been employed to predict the postoperative effective lens position and anticipate refractive outcomes, with the aim of minimizing predictive errors [9]. The traditional power calculation model relies on the axial length, corneal K value with only the anterior radius, and ACD for prediction [10]. Current IOL formulas include not only the three variables mentioned above but also lens thickness, central corneal thickness, and white-to-white corneal diameter. For example, the SRK/T formula is one of the most widely used methods for calculating the IOL [8]. In a study by Ota et al., which used the SRK/T formula to calculate the same type of EDOF IOL used in our study, 38–61% of the eyes were within 0.3 D of the prediction error. The discrepancy observed between this study and ours may be attributable to variations in the axial length between Ota et al.'s series (mean: 24.0 $\pm$ 1.3 mm, 25.4 $\pm$ 1.8 mm) and our dataset (mean: 25.6 $\pm$ 1.8 mm) [11]. In an investigation by Jeon et al., which used the SRK/T formula alongside newer formulas such as Barrett Universal II, Kane, and Olsen to calculate the Vivity EDOF IOLs (Alcon, Fort Worth, TX, USA) in patients with a mean axial length of 23.53 mm, a mean absolute prediction error of approximately 0.3 D was reported across all formulas, with the SRK/T formula exhibiting a larger standard deviation [12]. Despite the larger standard deviation, the SRK/T formula remains a reliable choice for calculating the EDOF IOL.

The consideration of not using presbyopia-correcting IOLs in patients with cataract who have a history of LASIK has been discussed in a previous review [13]. This discussion arises from the potential for greater residual refractive errors due to less predictable IOL power calculations compared with non-LASIK eyes. Such unpredictability can lead to unsatisfactory visual outcomes or necessitate re-enhancement procedures [13]. To address inaccuracies in corneal radius and keratometric measurements following refractive surgery procedures, several specialized IOL power calculation formulas have been introduced. Among these, the Haigis-L formula has gained popularity because it does not require previous refractive surgery data [14]. In the study by Abulafia et al., which examined patients with a history of LASIK or photorefractive keratectomy, the mean absolute prediction error was 0.68 $\pm$ 0.45 D via the Haigis-L method, 0.63 $\pm$ 0.48 D via the Shammas method, and 0.52 $\pm$ 0.43 D using the Barrett true-K formula [4]. These values were similar to the 0.65 $\pm$ 0.26 D observed in our study, which used the Haigis-L formula.

An interesting observation from our results pertains to the uncorrected distance visual acuity (UDVA) outcomes. Specifically, the LASIK group exhibited a mean UDVA of 0.300 $\pm$ 0.362 logMAR, which was notably worse than the 0.117 $\pm$ 0.156 logMAR observed in the non-LASIK group. The 95% confidence interval for the difference (0.0033 to 0.3585) encompassed the non-inferiority margin of 0.1 logMAR, indicating that the LASIK group could not be confirmed as non-inferior to the non-LASIK group in this parameter. While the regression analysis did not reveal a statistically significant difference between the groups after adjusting for confounders, this finding raises important considerations. One possible explanation is the presence of subtle corneal irregularities or higher-order aberrations (HOAs) induced by prior LASIK, which may impact the distance vision quality despite acceptable refractive outcomes. LASIK surgery, especially when performed years prior with older technologies, can result in postoperative corneal asphericity and induction of coma aberrations, both of which can degrade the image quality under scotopic conditions

and reduce the UDVA even when refraction is close to plano [15,16]. In contrast, non-LASIK eyes typically preserve a more regular corneal shape, which may contribute to sharper unaided distance vision following the EDOF IOL implantation. Additionally, the significantly longer axial lengths and flatter corneal curvatures seen in the LASIK group could have influenced the effective lens position (ELP) or created minor defocus, leading to slight reductions in the UDVA. Previous studies have shown that small prediction errors in ELP estimation can have a disproportionate impact on the UDVA, particularly in eyes with long axial lengths [17]. It was consistent with our study: in the regression analysis, after adjusting for confounders, the LASIK and non-LASIK groups had no difference in terms of UDVA, but the axial length had a significant influence on the UDVA. Our study also revealed that the LASIK group may have a better UNVA and a greater prediction error compared with the non-LASIK group, which could be proof that small prediction errors in the ELP estimation could cause more minor defocus toward the minus degree of refraction, which mimics mild myopic eyes and therefore cause lower UDVA and better UNVA in the LASIK group.

Our study has some limitations. First, our samples were retrospectively selected, presenting a limitation regarding sample size. The relatively small sample size, particularly in the LASIK group, may limit the generalizability of the results. Moreover, after matching the groups based on age and sex, most of the data could not be used. Second, differences in the preoperative demographics and clinical characteristics, including the axial length and ACD, between the groups could introduce potential errors in data interpretation. Third, the follow-up period of one month after surgery may be short for EDOF IOLs because EDOF IOLs may have better visual outcome after a longer follow-up period. Fourth, additional parameters that are important measures of visual quality and satisfaction, such as contrast sensitivity, wavefront aberration, and subjective questionnaires for photic phenomena, such as halo and glare, were excluded. Fifth, the SRK/T formula is a reliable choice for calculating the EDOF IOL; however, it is an early-generation formula. Now, we have more modern formulas which can improve the refraction prediction accuracy, such as the Barrett True-K formula. Due to the retrospective nature of the study, we could not assess the result by utilizing these modern formulas. Further prospective studies with larger cohorts, longer follow-up periods, modern formulas, and comprehensive assessments are warranted to validate our findings.

5. Conclusions

In conclusion, our study demonstrated that previous LASIK may have no discernible effect on the visual performance of presbyopia-correcting EDOF IOLs with respect to the absolute refractive error, UNVA, and UDVA. For further validation, longer follow-up and larger-scale or multicenter studies are required to ensure the robustness and generalizability of our results in diverse clinical settings.

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Informed Consent Statement: Patient consent was waived owing to the retrospective nature of the study.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ACD	Anterior chamber depth
CDVA	Corrected distance visual acuity
D	Diopter
EDOF	Extended depth of focus
IOLs	Intraocular lenses
K	Corneal curvature
LASIK	Laser-assisted in situ keratomileusis
logMAR	Logarithm of the minimum angle of resolution
SE	Spherical equivalent
SRK/T	Sanders Retzlaff Kraff/theoretical
UDVA	Uncorrected distance visual acuity
UNVA	Uncorrected near visual acuity

References

1. Kim, T.I.; Del Barrio, J.L.A.; Wilkins, M.; Cochener, B.; Ang, M. Refractive surgery. *Lancet* **2019**, *393*, 2085–2098. [CrossRef] [PubMed]
2. Mai, E.L.; Chang, C.-K.; Lee, C.-Y.; Lian, I.-B.; Chao, C.-C. Higher-order aberrations of topography-guided LASIK and wavefront-optimized LASIK in high- and low-myopic eyes: A non-randomized controlled trial. *J. Pers. Med.* **2023**, *13*, 399. [CrossRef] [PubMed]
3. Yeu, E.; Cuozzo, S. Matching the patient to the intraocular lens: Preoperative considerations to optimize surgical outcomes. *Ophthalmology* **2021**, *128*, e132–e141. [CrossRef] [PubMed]
4. Abulafia, A.; Hill, W.E.; Koch, D.D.; Wang, L.; Barrett, G.D. Accuracy of the Barrett True-K formula for intraocular lens power prediction after laser in situ keratomileusis or photorefractive keratectomy for myopia. *J. Cataract. Refract. Surg.* **2016**, *42*, 363–369. [CrossRef] [PubMed]
5. Walker, J. Non-inferiority statistics and equivalence studies. *BJA Educ.* **2019**, *19*, 267–271. [CrossRef] [PubMed]
6. Ang, M.; Tan, D.; Mehta, J.S. Small incision lenticule extraction (SMILE) versus laser in-situ keratomileusis (LASIK): Study protocol for a randomized, non-inferiority trial. *Trials* **2012**, *13*, 75. [CrossRef] [PubMed]
7. Day, A.C.; Burr, J.M.; Bunce, C.; Doré, C.J.; Sylvestre, Y.; Wormald, R.P.L.; Round, J.; McCudden, V.; Rubin, G.; Wilkins, M.R. Randomised, single-masked non-inferiority trial of femtosecond laser-assisted versus manual phacoemulsification cataract surgery for adults with visually significant cataract: The FACT trial protocol. *BMJ Open* **2015**, *5*, e010381. [CrossRef] [PubMed]
8. Kane, J.X.; Chang, D.F. Intraocular lens power formulas, biometry, and intraoperative aberrometry: A review. *Ophthalmology* **2021**, *128*, e94–e114. [CrossRef] [PubMed]
9. Shajari, M.; Sonntag, R.; Niermann, T.; Holland, D.; Kohnen, T.; Priglinger, S.; Mayer, W.J. Determining and comparing the effective lens position and refractive outcome of a novel rhexis-fixated lens to established lens designs. *Am. J. Ophthalmol.* **2020**, *213*, 62–68. [CrossRef] [PubMed]
10. Simpson, M.J. Pre-clinical estimation of the intraocular lens A-constant, and its relationship to power, shape factor, and asphericity. *Appl. Opt.* **2021**, *60*, 5662–5668. [CrossRef] [PubMed]
11. Ota, Y.; Bissen-Miyajima, H.; Nakamura, K.; Hirasawa, M.; Minami, K. Binocular visual function after staged implantation of extended-depth-of-focus intraocular lens targeting emmetropia and -0.5 diopter: A prospective comparison. *PLoS ONE* **2020**, *15*, e0238135. [CrossRef] [PubMed]
12. Jeon, S.; Taroni, L.; Lupardi, E.; Hoffer, K.J.; Fontana, L.; Schiano-Lomoriello, D.; Kwon, H.; Savini, G. Accuracy of nine formulas to calculate the powers of an extended depth-of-focus IOL using two SS-OCT biometers. *J. Refract. Surg.* **2023**, *39*, 158–164. [CrossRef] [PubMed]

13. Khor, W.B.; Afshari, N.A. The role of presbyopia-correcting intraocular lenses after laser in situ keratomileusis. *Curr. Opin. Ophthalmol.* **2013**, *24*, 35–40. [CrossRef] [PubMed]
14. Chen, X.; Yuan, F.; Wu, L. Metaanalysis of intraocular lens power calculation after laser refractive surgery in myopic eyes. *J. Cataract. Refract. Surg.* **2016**, *42*, 163–170. [CrossRef] [PubMed]
15. Marcos, S.; Barbero, S.; Llorente, L.; Merayo-Lloves, J. Optical response to LASIK surgery for myopia from total and corneal aberration measurements. *Investig. Ophthalmol. Vis. Sci.* **2001**, *42*, 3349–3356. [PubMed]
16. Feng, Z.; Wang, Q.; Du, C.; Yang, F.; Li, X. High-order aberration changes after femtosecond LASIK surgery in patients with high myopia. *Ann. Palliat. Med.* **2021**, *10*, 7689–7696. [CrossRef] [PubMed]
17. Yin, S.; Guo, C.; Qiu, K.; Ng, T.K.; Li, Y.; Du, Y.; Chen, B.; Wang, H.; Zhang, M. Assessment of the influence of keratometry on intraocular lens calculation formulas in long axial length eyes. *Int. Ophthalmol.* **2022**, *42*, 3211–3219. [CrossRef] [PubMed]

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Evaluation of the Choroidal Thickness and Retinal Nerve Fiber Layer Thickness in Patients with Vasovagal Syncope

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Abstract: Aim: The aim of this study was to evaluate choroidal and peripapillary retinal nerve fiber layer (RNFL) thicknesses in individuals with vasovagal syncope (VVS). **Method**: A total of 67 consecutive patients with VVS and 61 healthy control subjects were enrolled this study. The choroidal thickness (CT) at the fovea, the nasal to fovea thickness, and the temporal to fovea thickness were measured, alongside pRNLFT measurements assessed by swept-source optical coherence tomography (SS-OCT). **Results**: The mean foveal CT (408.7 ± 92.5 μm vs. 342.1 ± 60.2 μm, $p < 0.01$), the mean nasal CT (385.2 ± 88.3 μm vs. 329.2 ± 47.6 μm, $p < 0.001$), and the mean temporal CT (379.5 ± 51.6 μm vs. 321.48 ± 43.2 μm, $p < 0.03$) were statistically thicker in patients with VVS compared to the healthy controls. There was no statistically significant difference in the global pRNFLT measurements and all quadrants between the study groups. **Conclusions**: The CT in all regions was found to be thicker in patients with VVS compared to the healthy controls, while there were no differences in pRNFLT values. These results suggest that choroidal circulation might be affected by local neurotransmitter alterations in patients with VVS.

Keywords: choroidal thickness; optical coherence tomography; vasovagal syncope

1. Introduction

Syncope is a loss of consciousness defined by a loss of postural tone, amnesia, and rarely, the disruption of smooth muscle tonus, with a short duration and spontaneous complete recovery [1]. Vasovagal syncope (VVS) is a common form of syncope that originates from a sudden failure of the autonomic nervous system to sustain adequate vascular tone under orthostatic stress. It may be a compensatory maneuver to protect the central nervous system and heart from permanent ischemic damage. Even though VVS is commonly benign, repeated episodes could adversely affect quality of life and might increase the risk of major adverse events [2].

In vasovagal syncope, the cardioinhibitory pathway involves parasympathetic hypertonicity, which affects the sinoatrial and atrioventricular nodes and may cause a long asystole or nodal rhythm [3]. On the other hand, the vasodepressor efferent pathway causes a decrease in vascular sympathetic tone, leads to arterial and venous dilation, and results in a reduction in peripheral vascular resistance and arterial blood pressure. Consequently, blood is pooled in the lower extremities and/or in the splanchnic beds, and venous

return is reduced. As a result, stroke volume decreases, causing cerebral and systemic hypoperfusion [1,3,4].

As far as recent studies are concerned, the pathophysiology of VVS has still not been clearly clarified. It has been proposed that the development of VVS is associated with changes in hemodynamic parameters that result from variations in the plasma levels of vasoactive substances, such as nitric oxide, catecholamines, endothelin, prostanoids, or vasopressin, as part of the body's response to orthostatic stress [1,5]. Moreover, paradoxical peripheral vasodilation caused by endothelial dysfunction might be important in improper excessive hypotension during orthostatic stress in individuals with VVS. Factors like inflammation and oxidative stress have also been associated with impaired endothelial function in these patients [5,6].

The choroid has a highly vascular structure, and choroidal blood flow is crucial for the health of the retina, supplying oxygen and nutrients to its outer layers [7]. Neurogenic control significantly influences choroidal blood flow through the autonomic nervous system [8]. Various factors, including neurotransmitters and local metabolic signals, modulate these neurogenic controls [9]. This regulation is vital during changes in light conditions or visual tasks, ensuring efficient nutrient delivery and waste removal from retinal tissues. As the use of optical coherence tomography (OCT) technology, especially swept-source optical coherence tomography (SS-OCT) technology, has increased, non-invasive detailed evaluation of choroidal and retinal tissues has become possible [10].

In this study, we aimed to investigate whether dysregulation of the parasympathetic and/or sympathetic nervous system affects ocular structural measurements by evaluating choroidal thickness (CT) and peripapillary retinal nerve fiber layer thickness (pRNFLT) in individuals with VVS.

2. Materials and Methods

2.1. Patient Population

The data used in this study were acquired prospectively. This study was conducted in accordance with the principles outlined in the Declaration of Helsinki and received approval from the institutional ethics committee. Patients aged between 18 and 60 years who exhibited a history of recurrent episodes of apparent syncope, having experienced more than two such occurrences in the past six months, were included in the study. The inclusion criteria included (1) a positive drug-free head-up tilt table test (HUTT), in which syncope was deemed to have reproduced spontaneous symptoms; (2) absence of left ventricular systolic dysfunction and valve insufficiency and/or stenosis over a mild degree (estimated ejection fraction > 50% using transthoracic echocardiography); (3) absence of obesity (body mass index < 30), hematologic or biochemical abnormalities, or use of drugs known to predispose to orthostatic hypotension. The exclusion criteria can be listed as (1) patients not meeting the above criteria; (2) individuals with a history of systemic diseases that might affect CT and pRNFLT, such as systemic arterial hypertension, diabetes mellitus, and rheumatologic diseases based on self-reported medical history; (3) eyes with high myopia (>6D); (4) advanced-level cataracts, age-related macular degeneration, or a history of retinal vascular disease; (5) patients with abnormal intraocular pressure (IOP), glaucoma, or ocular hypertension; (6) patients with a history of intraocular surgery and tachycardia or autonomic dysfunction associated with neurologic disorders including Parkinsonism, autonomic failure, peripheral neuropathy, or postural tachycardia syndrome.

2.2. Head-Up Tilt Table Testing Protocol

All patients stopped taking cardioactive medication for at least five half-lives prior to HUTT. Each patient was gently secured to the tilt table, ensuring appropriate positioning

to allow for the use of a footboard for weight bearing. HUTT was performed in the morning after a 12 h overnight fasting period. A minimum 15 min equilibration time, with the patients resting in a supine position in a dark, quiet environment, was allowed before the commencement of baseline recordings for the HUTT procedure. In our standard protocol, there were 15 min of rest followed by 45 min (or until the onset of symptoms) at a 70-degree angle [11]. The predictive value of HUTT was enhanced with sublingual glyceryl trinitrate [11,12]. Systemic arterial blood pressure and heart rate were continuously recorded, automatically calculating all the R-R intervals throughout the test using a non-invasive, beat-by-beat monitor CardioLab® (GE Healthcare, Chicago, IL, USA), and data were collected by a computer that processed them using ad hoc software.

2.3. Ophthalmic Examinations

All participants in the study underwent an extensive and thorough ophthalmic assessment, which included a variety of tests designed to evaluate different aspects of visual function and ocular health. The evaluation began with an assessment of best-corrected visual acuity, which was performed using the Snellen chart. Additionally, intraocular pressure was measured employing the Goldmann applanation tonometry technique, a reliable method for gauging the pressure within the eye.

The examination also encompassed slit lamp biomicroscopy, a diagnostic procedure that allows for detailed observation of the anterior segment of the eye, and a dilated fundus examination to facilitate a comprehensive view of the retina and other posterior structures. To measure the axial length of the eye, the IOL Master 500 (manufactured by Carl Zeiss Meditec Inc., based in Jena, Germany) was utilized, providing precise measurements essential for evaluating ocular dimensions.

Furthermore, the choroidal thickness (CT) and the peripapillary retinal nerve fiber layer thickness (pRNFLT) were quantified using the swept-source optical coherence tomography (SS-OCT) system, specifically the Topcon Triton model from Japan. It is worth mentioning that all measurements and assessments were consistently carried out by the same trained technician during similar time frames, specifically between 9:00 a.m. and 12:00 p.m., to minimize potential variabilities. HUTT and OCT procedures were not performed on the same day.

In determining the choroidal thickness, which was defined as the axial distance from the retinal pigment epithelium (RPE) to the outer interface of the choroid/sclera, evaluations were conducted by a single ophthalmologist (H.S.K). When H.S.K performed the OCT evaluation, she did not know whether the patient had VVS or not, and the study was single-blind. The CT measurements were specifically taken in the subfoveal region, as well as at distances of 1500 μm temporally and 1500 μm nasally from the foveal region. For the assessment of the pRNFLT, the measurements were obtained using OCT by employing a circular scan with a diameter of 3.46 mm that was centered on the optic disk.

The resulting data provided pRNFLT values for each of the four quadrants—superior (S), inferior (I), nasal (N), and temporal (T)—as well as the global mean values by using software program. It is important to note that the statistical analyses were conducted using the average values derived from both eyes of each participant, allowing for a comprehensive evaluation of the visual and ocular health indicators across the sample. Figures 1 and 2 demonstrate the OCT and pRNLFT measurements for participants with VVS and a healthy control.

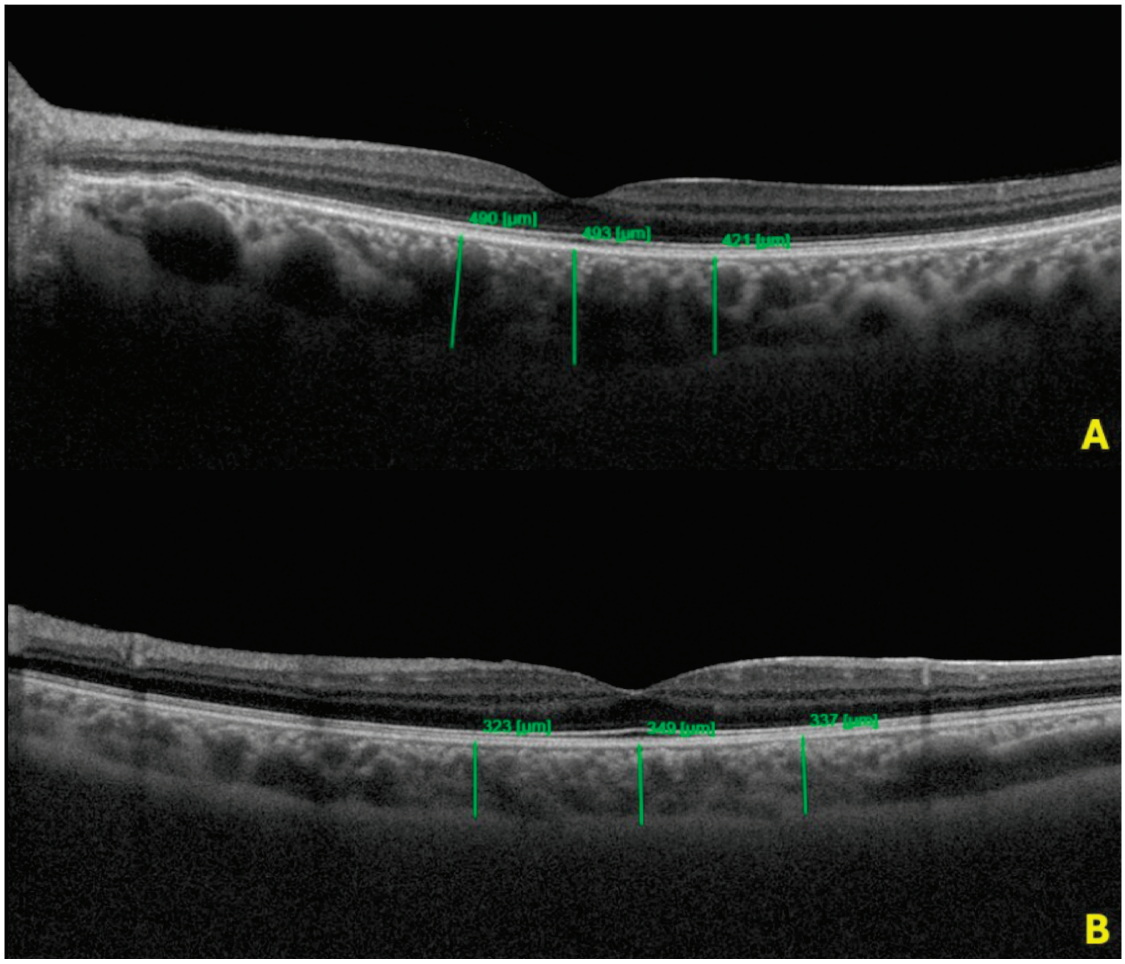

Figure 1. (**A**): Increased CT in the fovea, temporal to fovea, and nasal to fovea regions in a patient with VVS; (**B**): CT in the fovea, temporal to fovea, and nasal to fovea regions in a healthy control.

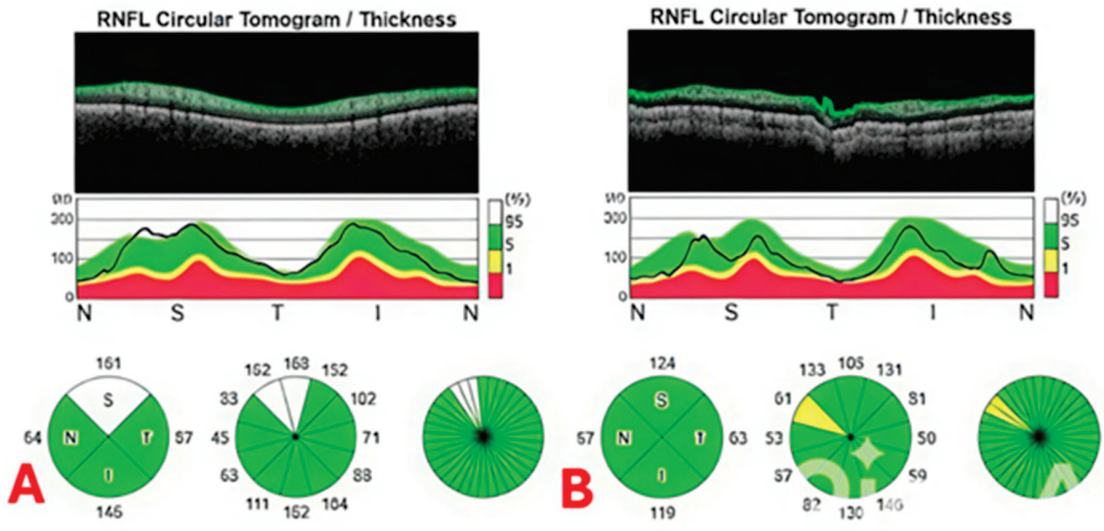

Figure 2. (**A**): pRNFL images of a healthy control; (**B**): pRNFL images of a patient with VVS.

2.4. Statistical Analysis

Statistical analyses were conducted utilizing SPSS software, specifically version 21, provided by SPSS Inc., located in Chicago, IL, USA. Continuous variables in the study were presented as mean values accompanied by their corresponding standard deviation (SD). To

ensure the validity of the assumed linear relationships and the principle of homoscedasticity across the datasets, we evaluated these assumptions through the examination of standardized residuals plots. Furthermore, the assumption of normality for the dependent variable was assessed using the Kolmogorov–Smirnov statistical criterion.

In addition, to detect significant differences among categorical variables, the Chi-square test was employed. As all continuous variables demonstrated a normal distribution, statistical comparisons of quantitative data were carried out using the unpaired-sample *t*-test method. It is important to note that differences were regarded as statistically significant when the *p*-value was less than 0.05. This level of significance indicates that we could reject the null hypothesis in favor of the alternative hypothesis, suggesting that there is a meaningful difference worthy of further discussion and analysis.

3. Results

A total of 67 patients with VVS were included in this study, and their data were compared with those of 61 age- and sex-matched healthy controls. The mean age of patients with VVS was 35.2 ± 6.3 years, while that of the control group subjects was 36.3 ± 7.4 years. Demographic and clinical information about the patients and control subjects is summarized in Table 1. No significant differences were noted in the values of intraocular pressure, axial length, systemic arterial blood pressure measurements, smoking status, age, and sex between patients with VVS and the healthy controls.

Table 1. Demographic and clinical data of the study population.

	Patients with VVS (*n* = 67)	**Healthy Controls (*n* = 61)**	***p* Value**
Age (years)	35.2 ± 6.3	36.3 ± 7.4	0.13
Gender (Female%)	42(62.7%)	35(57.4%)	0.08
Body mass index (kg/m^2)	22.4 ± 5.6	21.5 ± 3.9	0.23
Smoking	14(20.9%)	12(19.6%)	0.47
SRE (D)	+0.71 ± 0.2	+0.58 ± 0.3	0.55
Axial length (mm)	23.08 ± 0.4	22.98 ± 0.3	0.67
Intraocular pressure (mm Hg)	16.25 ± 5.3	18.04 ± 4.8	0.25
Systolic BP (mmHg)	108.3 ± 7.2	123.4 ± 9.3	0.22
Diastolic BP (mmHg)	76.7 ± 4.7	79.9 ± 5.9	0.12
Mean BP (mmHg)	86.7 ± 5.5	94.3 ± 6.9	0.19

Note: data are presented as mean ± standard deviation while categorical variables are expressed as percentages. Abbreviations: BP: blood pressure; SRE: spherical refractive error.

In the CT evaluation, a statistically significant difference was noted between patients with VVS and the healthy controls. The mean foveal CT (408.7 ± 92.5 μm vs. 342.1 ± 60.2 μm, $p < 0.001$), mean nasal CT (385.2 ± 88.3 μm vs. 329.2 ± 47.6 μm, $p < 0.001$), and mean temporal CT (379.5 ± 91.6 μm vs. 321.48 ± 43.2 μm, $p < 0.001$) were statistically thicker in patients with VVS compared to the healthy controls. There was no statistically significant difference in pRNFLT measurements between patients with VVS and the healthy controls. Table 2 summarizes the comparison of SS-OCT measurements between the study groups.

Table 2. Summary of statistical analyses for comparison of SFCT, pRNFLT (average), and quadrants of pRNFLT.

	Participants with VVS (n = 67)	Healthy Controls (n = 61)	p Value
Foveal CT (μm)	408.7 ± 92.5	342.1 ± 60.2	**<0.001**
Nasal CT (μm)	385.2 ± 98.3	329.2 ± 47.6	**<0.001**
Temporal CT (μm)	379.5 ± 91.6	321.48 ± 43.2	**<0.001**
Global pRNFLT (μm)	106.7 ± 7.4	108.2 ± 6.2	0.11
Inferior pRNFLT (μm)	128.6 ± 8.3	129.2 ± 8.9	0.41
Superior pRNFLT (μm)	127.6 ± 7.9	129.8 ± 8.4	0.31
Nasal pRNFLT (μm)	86.5 ± 6.7	87.6 ± 6.3	0.24
Temporal pRNFLT (μm)	84.4 ± 6.5	86.2 ± 6.8	0.26

Note: data are presented as mean ± standard deviation. Bold values indicate statistical significance $p < 0.05$. Abbreviations: CT: choroidal thickness; pRNFLT: peripapillary retinal nerve fiber layer thickness; SFCT: subfoveal choroidal thickness; VVS: vasovagal syncope.

4. Discussion

In this study, we evaluated the CTs and pRNFLT in participants with VVS and compared these values with those of healthy controls. The main outcomes of this study are as follows: (1) the mean CT in all regions was significantly thicker in participants with VVS compared to the control group. (2) There were no significant differences in all pRNFLT measurements between study groups.

Both sympathetic and parasympathetic pathways contribute to choroidal blood flow regulation, with sympathetic fibers generally causing vasoconstriction and parasympathetic fibers promoting vasodilation [7]. Parasympathetic stimulation, particularly through acetylcholine release, promotes vasodilation, increasing blood flow to meet the metabolic demands of the retina, especially during high visual activity. In contrast, sympathetic activation, associated with norepinephrine, causes vasoconstriction, reducing blood flow [13]. Various neurotransmitters also influence choroidal blood flow regulation. For example, nitric oxide promotes vasodilation, while other molecules like vasoactive intestinal peptide and substance p additionally modulate vessel diameter [7,13].

The dysregulation of the autonomous nerve system and the alteration of neurotransmitters have crucial roles in VVS pathogenesis. Jardine at el. showed parasympathetic hypertonicity in participants with VVS [14]. Aksu et al. showed that low endothelin-1 levels, which is a pro-inflammatory vasoconstrictor peptide, are associated with a higher risk of VVS. In addition, neuropeptide Y may also play a role in VVS by increasing peripheral vascular resistance and decreasing cardiac output during orthostatic regulation [15]. Liao et al. revealed that neuropeptide Y plasma levels are significantly reduced in individuals with VVS [16]. Gallegos et al. showed that nitric oxide levels are increased in VVS, probably due to high catecholamine plasma levels. Nitric oxide synthase could be activated by $\beta-2$ receptors, increasing the release of nitric oxide from the vascular endothelium and leading to excessive vasodilation [17]. Endothelin-1 was found to be negatively correlated with choroidal blood flow, and endothelin-1 increases vasoconstriction in choroid and retinal blood. [18,19]. Some studies conducted with animals have shown that choroidal vasodilation and choroidal thickness may increase due to increased nitric oxide [20,21]. In this study, we found that the CT in the fovea, nasal to the fovea, and temporal to the fovea regions was statistically significantly thicker than in the healthy controls. Increased CT in participants with VVS might be related to the increase in parasympathetic activity and the change in neurotransmitters.

Thicker CT is linked to several ocular diseases and serves as an important biomarker for diagnosis and management [22,23]. Pachychoroid spectrum diseases, age-related macular degeneration, and some uveitic diseases are among the main diseases in which CT is important [22,24]. Patients with central serous chorioretinopathy have an especially high CT and type A personality traits [25–27]. It is known from previous studies that participants with VVS have stressed personality structures [28,29]. Therefore, participants with VVS may also be prone to ocular diseases such as central serous chorioretinopathy due to their high CT. Further studies may clarify this relationship.

The pRNFL is the region surrounding the optic disk where nerve fibers from the retina converge to form the optic nerve and is supplied by the central retinal artery [30]. This layer consists primarily of the axons of ganglion cells and is essential for transmitting visual information from the retina to the brain. The health of the pRNFL is vital for visual function, as changes in its thickness can indicate various ocular diseases, especially glaucoma [31,32]. Thinning of the pRNFL is often a sign of damage to the optic nerve and progressive loss of ganglion cells, necessitating careful monitoring [31]. We compared the pRNFLT values of participants with VVS with healthy controls, and we did not find any differences between participants with VVS and the healthy controls. It is known that there are distinct differences between choroidal and retinal artery blood flow [33]. Retinal blood flow is tightly regulated by local metabolic demands, allowing it to adjust to the oxygen needs of the inner retina. In contrast, choroidal blood flow is influenced more by systemic factors rather than local needs, leading to less autoregulation [33–35]. For this reason, while we detected changes in CTs in participants with VVS, we may not have detected changes in pRNFLT values, which indicate retinal artery blood flow.

This study has some limitations. For instance, the size of the study groups was relatively small. Moreover, a manual method was used to measure CTs instead of using an automated software program, which is more reliable. We evaluated only choroidal thickness and no other parameters such as the choroidal vascular index [36]. Patients' anxiety status and/or personality evaluation were not recorded. In this study, we did not evaluate the neurotransmitter levels in plasma and/or serum. In the HUTT protocol, we did not use the Vasovagal Syncope International Study (BASIS) criteria for the subgroup analysis of the participants with VVS. The VASIS classification includes the following categories: mixed (VASIS-1), cardioinhibition (VASIS-2A), severe cardioinhibition/asystole (VASIS-2B), and pure vasodepression (VASIS-3). There may be differences in the subgroup analysis of the participants in terms of CT measurements [37].

5. Conclusions

In conclusion, we showed that participants with VVS exhibited greater CT across all measured regions when compared to healthy controls. Conversely, there were no significant variations observed in the thickness of the pRNFL. These findings imply that the choroidal blood flow could be influenced by changes in local neurotransmitter levels in individuals suffering from VVS. This interplay underscores the connection between cardiovascular health and CT alterations, suggesting a need for monitoring in individuals with frequent syncopal episodes to prevent complications and maintain ocular health. Further investigation into these dynamics may yield insights beneficial for clinical practice regarding VVS and ocular health.

Author Contributions: H.B.I.: writing—original draft, software, investigation, formal analysis, data curation, conceptualization. B.G.K.: writing—original draft, validation, formal analysis, data curation, conceptualization. G.A.: writing—original draft, validation, resources, methodology, investigation, formal analysis, data curation. S.D.: writing—original draft, validation, resources, methodology, investigation, formal analysis, data curation. H.S.K.: writing—original draft, validation, formal

analysis, data curation, conceptualization. M.V.Y.: data curation, reviewing and editing. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the ethics committee of the Marmara University School of Medicine (protocol code: 11.2023.1269 and date of approval: 11 December 2023).

Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: Certain confidentiality constraints prevent us from disclosing the underlying database used in this study.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Longo, S.; Legramante, J.M.; Rizza, S.; Federici, M. Vasovagal syncope: An overview of pathophysiological mechanisms. *Eur. J. Intern. Med.* **2023**, *112*, 6–14. [CrossRef] [PubMed]
2. Sandhu, R.K.; Raj, S.R.; Thiruganasambandamoorthy, V.; Kaul, P.; Morillo, C.A.; Krahn, A.D.; Guzman, J.C.; Sheldon, R.S. Canadian Cardiovascular Society Clinical Practice Update on the Assessment and Management of Syncope. *Can. J. Cardiol.* **2020**, *36*, 1167–1177. [CrossRef]
3. van Dijk, J.G.; van Rossum, I.A.; Thijs, R.D. Timing of Circulatory and Neurological Events in Syncope. *Front. Cardiovasc. Med.* **2020**, *7*, 36. [CrossRef] [PubMed]
4. van Dijk, J.G.; van Rossum, I.A.; Thijs, R.D. The pathophysiology of vasovagal syncope: Novel insights. *Auton. Neurosci.* **2021**, *236*, 102899. [CrossRef]
5. Pachon-M, J.C. Neurocardiogenic syncope: Pacemaker or cardioneuroablation? *Heart Rhythm.* **2020**, *17*, 829–830. [CrossRef] [PubMed]
6. Pietrucha, A.Z. Endothelial function in vasovagal syncope. [published correction appears]. *Expert. Rev. Cardiovasc. Ther.* **2014**, *12*, 1387–1389, Erratum in *Expert Rev. Cardiovasc. Ther.* **2015**, *13*, 455–484. https://doi.org/10.1586/14779072.2015.1024484. [CrossRef]
7. Reiner, A.; Fitzgerald, M.E.C.; Del Mar, N.; Li, C. Neural control of choroidal blood flow. *Prog. Retin. Eye Res.* **2018**, *64*, 96–130. [CrossRef]
8. Motamed Shariati, M.; Khazaei, S.; Yaghoobi, M. Choroidal vascularity index in health and systemic diseases: A systematic review. *Int. J. Retin. Vitr.* **2024**, *10*, 87–96. [CrossRef]
9. Shin, D.Y.; Hong, K.E.; Lee, N.Y.; Park, C.K.; Park, H.Y.L. Association of choroidal blood flow with autonomic dysfunction in patients with normal tension glaucoma. *Sci. Rep.* **2022**, *12*, 5136–5142. [CrossRef]
10. Singh, S.R.; Vupparaboina, K.K.; Goud, A.; Dansingani, K.K.; Chhablani, J. Choroidal imaging biomarkers. *Surv. Ophthalmol.* **2019**, *64*, 312–333. [CrossRef]
11. Aponte-Becerra, L.; Novak, P. Tilt Test: A Review. *J. Clin. Neurophysiol.* **2021**, *38*, 279–286. [CrossRef] [PubMed]
12. van Zanten, S.; Sutton, R.; Hamrefors, V.; Fedorowski, A.; de Lange, F.J. Tilt table testing, methodology and practical insights for the clinic. *Clin. Physiol. Funct. Imaging* **2024**, *44*, 119–130. [CrossRef]
13. Wei, X.; Balne, P.K.; Meissner, K.E.; Barathi, V.A.; Schmetterer, L.; Agrawal, R. Assessment of flow dynamics in retinal and choroidal microcirculation. *Surv. Ophthalmol.* **2018**, *63*, 646–664. [CrossRef] [PubMed]
14. Piotrowski, R.; Baran, J.; Sikorska, A.; Krynski, T.; Kulakowski, P. Cardioneuroablation for Reflex Syncope: Efficacy and Effects on Autonomic Cardiac Regulation-A Prospective Randomized Trial. *JACC Clin. Electrophysiol.* **2023**, *9*, 85–95. [CrossRef] [PubMed]
15. Aksu, T.; Davila, A.; Gupta, D. The "heart brain" and neuromodulation for vasovagal syncope. *Auton. Neurosci.* **2021**, *236*, 102892. [CrossRef]
16. Liao, Y.; Xu, W.R.; Li, H.X.; Tang, C.S.; Jin, H.F.; Du, J.B. Plasma Neuropeptide Y Levels in Vasovagal Syncope in Children. *Chin. Med. J.* **2017**, *130*, 2778–2784. [CrossRef]
17. Gallegos, A.; Márquez-Velasco, R.; Allende, R.; Gómez-Flores, J.R.; Cázares-Campos, I.; González-Hermosillo, A.; Bojalil, R.; Cárdenas, M.; Márquez, M.F. Serum concentrations of nitric oxide and soluble tumor necrosis factor receptor 1 (sTNFR1) in vasovagal syncope: Effect of orthostatic challenge. *Int. J. Cardiol.* **2013**, *167*, 2321–2322. [CrossRef]
18. Hétu, S.; Pouliot, M.; Cordahi, G.; Couture, R.; Vaucher, E. Assessment of retinal and choroidal blood flow changes using laser Doppler flowmetry in rats. *Curr. Eye Res.* **2013**, *38*, 158–167. [CrossRef]
19. Finzi, A.; Cellini, M.; Strobbe, E.; Campos, E.C. ET-1 plasma levels, choroidal thickness and multifocal electroretinogram in retinitis pigmentosa. *Life Sci.* **2014**, *118*, 386–390. [CrossRef]

20. Li, C.; Fitzgerald, M.E.; Del Mar, N.; Reiner, A. Stimulation of Baroresponsive Parts of the Nucleus of the Solitary Tract Produces Nitric Oxide-mediated Choroidal Vasodilation in Rat Eye. *Front. Neuroanat.* **2016**, *10*, 94. [CrossRef] [PubMed]
21. Erdinest, N.; London, N.; Ovadia, H.; Levinger, N. Nitric Oxide Interaction with the Eye. *Vision* **2021**, *5*, 29. [CrossRef] [PubMed] [PubMed Central]
22. Kim, Y.H.; Oh, J. Choroidal Thickness Profile in Chorioretinal Diseases: Beyond the Macula. *Front. Med.* **2021**, *8*, 797428. [CrossRef]
23. Sinawat, S.; Thongmee, W.; Sanguansak, T.; Laovirojjanakul, W.; Sinawat, S.; Yospaiboon, Y. Oral Spironolactone versus Conservative Treatment for Non-Resolving Central Serous Chorioretinopathy in Real-Life Practice. *Clin. Ophthalmol.* **2020**, *14*, 1725–1734. [CrossRef]
24. Steiner, M.; Esteban-Ortega, M.D.M.; Muñoz-Fernández, S. Choroidal and retinal thickness in systemic autoimmune and inflammatory diseases: A review. *Surv. Ophthalmol.* **2019**, *64*, 757–769. [CrossRef] [PubMed]
25. Mathews, R.; Horo, S.; Jose, D.; Kavalakatt, J.A.; J, G.R.; John, S.S. Role of Psychological Stress and Choroidal Thickness in Central Serous Chorioretinopathy. *Nepal. J. Ophthalmol.* **2023**, *15*, 36–46. [CrossRef]
26. Mukherji, S.; Karmakar, S.; Dasgupta, S. Association of Central serous chorioretinopathy with type of personality, anxiety and depression. *Indian. J. Ophthalmol.* **2024**, *72*, S60–S65. [CrossRef] [PubMed]
27. Mansour, A.M.; Koaik, M.; Lima, L.H.; Casella, A.M.B.; Uwaydat, S.H.; Shahin, M.; Tamim, H.; Sanchez-Ruiz, M.J.; Mansour, H.A.; Dodwell, D. Physiologic and Psychologic Risk Factors in Central Serous Chorioretinopathy. *Ophthalmol. Retin.* **2017**, *1*, 497–507. [CrossRef]
28. Abuzainah, B.; Gutlapalli, S.D.; Chaudhuri, D.; Khan, K.I.; Al Shouli, R.; Allakky, A.; Ferguson, A.A.; Khan, A.I.; Hamid, P. Anxiety and Depression as Risk Factors for Vasovagal Syncope and Potential Treatment Targets: A Systematic Review. *Cureus* **2022**, *14*, e32793. [CrossRef]
29. Ng, J.; Sheldon, R.S.; Ritchie, D.; Raj, V.; Raj, S.R. Reduced quality of life and greater psychological distress in vasovagal syncope patients compared to healthy individuals. *Pacing Clin. Electrophysiol.* **2019**, *42*, 180–188. [CrossRef]
30. Zabel, P.; Kałużny, J.J.; Wiłkość-Dębczyńska, M.; Gębska-Tołoczko, M.; Suwała, K.; Kucharski, R.; Araszkiewicz, A. Peripapillary Retinal Nerve Fiber Layer Thickness in Patients with Alzheimer's Disease: A Comparison of Eyes of Patients with Alzheimer's Disease, Primary Open-Angle Glaucoma, and Preperimetric Glaucoma and Healthy Controls. *Med. Sci. Monit.* **2019**, *25*, 1001–1008. [CrossRef] [PubMed] [PubMed Central]
31. Rao, H.L.; Pradhan, Z.S.; Suh, M.H.; Moghimi, S.; Mansouri, K.; Weinreb, R.N. Optical Coherence Tomography Angiography in Glaucoma. *J. Glaucoma* **2020**, *29*, 312–321. [CrossRef] [PubMed] [PubMed Central]
32. Deshpande, G.A.; Gupta, R.; Bawankule, P.; Raje, D.; Chakraborty, M. Evaluation of ganglion cell-inner plexiform layer thickness in the diagnosis of preperimetric glaucoma and comparison to retinal nerve fiber layer. *Indian J. Ophthalmol.* **2021**, *69*, 1113–1119. [CrossRef]
33. Benagiano, V.; Rizzi, A.; Sannace, C.; Alessio, G.; Ribatti, D.; Dammacco, R. Aqueous humor as eye lymph: A crossroad between venous and lymphatic system. *Exp. Eye Res.* **2024**, *243*, 109904. [CrossRef]
34. Brylla, E.; Tscheudschilsuren, G.; Santos, A.N.; Nieber, K.; Spanel-Borowski, K.; Aust, G. Differences between retinal and choroidal microvascular endothelial cells (MVECs) under normal and hypoxic conditions. *Exp. Eye Res.* **2003**, *77*, 527–535. [CrossRef]
35. Vader, M.J.C.; Habani, Y.I.; Schlingemann, R.O.; Klaassen, I. miRNA Levels as a Biomarker for Anti-VEGF Response in Patients with Diabetic Macular Edema. *J. Pers. Med.* **2021**, *11*, 1297. [CrossRef] [PubMed]
36. Hwang, S.; Kang, M.H.; Seong, M.; Cho, H.; Shin, Y.U. Swept-Source OCT Angiography Features in Patients after Macular Hole Surgery. *J. Pers. Med.* **2022**, *12*, 1493. [CrossRef]
37. Sutton, R.; Brignole, M.; Menozzi, C.; Raviele, A.; Alboni, P.; Giani, P.; Moya, A. Dual-chamber pacing in the treatment of neurally mediated tilt-positive cardioinhibitory syncope: Pacemaker versus no therapy: A multicenter randomized study. The Vasovagal Syncope International Study (VASIS) Investigators. *Circulation* **2000**, *102*, 294–299. [CrossRef]

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Diabetic Macular Edema in the Western Part of Romania: Screening to Improve Patient Outcomes

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Abstract: Background: Diabetes mellitus (DM) is a global healthcare concern with a rising prevalence. Patients with DM have a severely diminished quality of life due to the extensive range of connected complications. One of the most impactful diabetes-associated pathologies is diabetic macular edema (DME), as it is a major cause of blindness globally. Patients with DME present many concomitant diseases that influence their prognosis. The present research seeks to describe the most frequent DME-related comorbidities. **Method**: This study enrolled 105 participants previously diagnosed with type 1 DM (T1DM) or type 2 DM (T2DM) (77 presenting with DME), who were evaluated regarding other associated comorbidities. **Results**: Patients in the DME group presented a median age of 65, with a mean disease duration of 15 years and inadequate glycemic control, reflected by a mean HbA1c of 7.5%. All patients presented at least one comorbidity, with hypertension (100%) and dyslipidemia (62.3%) being the most prevalent. Spearman analysis revealed a statistically significant correlation between DME and diabetes duration ($p = 0.01$), proliferative diabetic retinopathy ($p = 0.004$), and chronic kidney disease ($p = 0.034$). **Conclusions**: Patients with DME often present multiple comorbidities that must be screened for and addressed through a multidisciplinary approach.

Keywords: diabetes mellitus; diabetic macular edema; health status; comorbidities; multidisciplinary; personalized medicine

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disease characterized by continuous hyperglycemia. According to the International Diabetes Federation's (IDF) 10th edition, the worldwide prevalence of diabetes is rising at an alarming rate, posing a significant challenge to public health [1]. DM is a leading contributor to major health issues, including cardiovascular diseases (CVD) such as acute myocardial infarction and strokes, as well as other severe complications like blindness and chronic kidney disease (CKD) [2].

Diabetic eye disease comprises several pathological changes, which include, among others, diabetic retinopathy (DR), diabetic macular edema (DME), glaucoma, cataracts, and even changes involving the ocular surface [3].

DME, with a continuously rising prevalence, is one of the most serious complications related to DM, as it is one of the leading causes of vision impairment worldwide [4]. DME can occur in any stage of DR, and its presence requires prompt and decisive therapeu-

tic intervention in order to prevent progression and irreversible retinal damage while simultaneously lowering the risk of blindness in patients with DM [5].

DME is characterized by retinal thickening, mostly in the outer and inner plexiform layers, mainly determined by a breakdown of the blood–retinal barrier [6]. This abnormal accumulation of fluid, lipids, and proteins involves the macular area and frequently impacts the fovea, directly leading to decreased visual acuity among patients with DM [2].

DME prevalence varies significantly, ranging from 4.2% to 14.3% in individuals with T1DM and from 1.4% to 5.57% in those with T2DM [7,8]. The high prevalence of DME not only severely affects patients' quality of life but also places a heavy economic overload on healthcare systems worldwide [2].

DME has a multifactorial nature, with a wide range of factors involved in its development. Gender, ethnicity, diabetes type/duration, or genetic factors represent unmodifiable factors, while hypertension (HTN), dyslipidemia, obesity, and chronic kidney disease are the most frequent pathologies linked to DME. Last but not least, habits like smoking or alcohol consumption also have a negative impact on DME evolution [9].

It is well known that patients with DM in general, not only those with DME, exhibit a higher risk of associated health problems than those who do not have DM. Approximately 60–70% of individuals with diabetes have at least one comorbidity, while 25–30% already present with more than two comorbidities at the time of their DM diagnosis. However, in everyday clinical practice, when these patients are screened for diabetes-related ocular complications, overall health status might be somewhat disregarded, resulting in overlooking potential factors that impact the development of these complications, especially DME. Many patients with DM, even those recently diagnosed, may already present chronic complications due to the prolonged metabolic dysregulation that often precedes the formal diagnosis of DM. For example, DM has been associated with changes in intraocular pressure (IOP). A recent study found that diabetes progression and altered glycemic control are directly linked to increased IOP before resulting in glaucoma [10]. Therefore, a thorough general and ophthalmologic evaluation is mandatory in all diabetic patients [11].

Regarding the systemic management of DME and other diabetes-related complications, the general consensus among healthcare professionals still involves glycemic control. However, considering the complex health status of patients with DM and the multiple risk factors involved in DME development, additional measures must be taken, such as strict blood pressure control, lowering lipid levels, and the proper management of obesity and renal status [12]. Last but not least, smoking and alcohol consumption cessation must be recommended in all patients with DM, despite the degree of retinal complications. These suggest that improving outcomes for patients with DME may require a broader approach, incorporating the investigation of additional risk factors and innovative treatment strategies beyond glycemic management alone [13].

The aim of this research was to evaluate the general health status of patients with DME, focusing on the diseases most frequently related to both DME and DM, in general, in order to raise awareness regarding the need for more stringent healthcare management of these patients, while also observing which are the most important risk factors implied in the appearance and evolution of DME, in order to more promptly screen and take preventative measures to ensure the prevention of blindness.

2. Materials and Methods

2.1. Study Design and Patient Selection

In this retrospective cross-sectional study, we included patients previously diagnosed with either T1DM or T2DM. All the patients took part in their prescheduled appointments

at the Diabetes Care Center within the "Pius Brinzeu" Emergency County Hospital in Timisoara, Romania.

We examined the medical records of 227 patients from the Diabetes Center between 16 July 2024 and 6 August 2024, and we enrolled a total number of 105 patients (55 male and 50 female subjects). 122 patients declined to participate in this research or did not comply with the study's eligibility criteria. The inclusion criteria comprised patients above 18 years of age with a prior DM diagnosis (either T1DM or T2DM). Exclusion criteria included other DM subtypes (gestational and secondary DM), significant cognitive impairment or other neuro-psychiatric disorders that prevented patients from giving informed consent, comorbidities that necessitated hospitalization during our study, institutionalized patients, and patients who presented any ocular pathologies that would interfere with a proper retinal examination (advanced cataracts and significant vitreous hemorrhage). Other retinal diseases that could interfere with an adequate classification of DME and DR by altering the examined retinal structures (severe myopia, detachment of the retina, or significant macular pucker) were also excluded from this research.

The protocol for the present study was approved by the Ethics Committee of the "Pius Brinzeu" Emergency County Hospital, Timisoara, Romania (approval no. 472/15.07.2024), adhering to the principles outlined in the Declaration of Helsinki, revised in 2013, and its subsequent amendments.

2.2. Data Collection and Medical Assessment

The following patient data were retrieved: gender, age, weight and height, diabetes type, disease duration, current medication, comorbidities, and smoking status. Additionally, a complete fundus examination was performed by a trained ophthalmologist, using a Topcon SL-D2 biomicroscope.

The assessment of biological parameters comprised a complete blood panel for all patients during their visit at the Diabetes Care Center. Diagnosis of DM was previously established through the presence of a fasting plasma glucose level > 7.0 mmol/L (126 mg/dL), 2 h post-load plasma glucose > 11.1 mmol/L (200 mg/dL), or HbA1c level above 6.5% (48 mmol/mol). Disease duration refers to the number of years from the initial DM diagnosis to the date of inclusion in the present research. Weight and height were measured for all participants, and the Body Mass Index (BMI) was calculated using the metric system. Following the World Health Organization (WHO) criteria regarding obesity, patients with BMI values above 25 kg/m^2 were considered overweight, while those with BMI equal to or above 30 kg/m^2 were diagnosed with different degrees of obesity. Blood pressure was also evaluated in all patients with the aid of an aneroid sphygmomanometer and hypertension was diagnosed in the presence of a systolic blood pressure value above or equal to 140 mmHg and a diastolic blood pressure above or equal to 90 mmHg. Meanwhile, CVD presence was derived from patients' medical history and referred to either a previous diagnosis of coronary heart disease or cerebrovascular disease. Diabetic neuropathy was evaluated by performing nerve conduction velocity (NCV), with values less than 40 m/s being considered pathological, as well as with the usage of the Michigan Neuropathy Screening Instrument (MNSI) with resulting clinical scores above 2.5, questionnaire scores above 7, or overall scores over 9.5. Chronic kidney disease (CKD) was determined at values below 60 mL/min of the creatinine-based glomerular filtration rate (eGFR) estimate. Dyslipidemia was diagnosed based on lipid panel values. Smoking habits were self-reported.

A board-certified ophthalmologist performed an extensive fundus examination after prior pupil dilation on all patients with a Topcon SL-D2 biomicroscope (Tokyo, Japan). Cataract diagnosis or prior cataract surgery with intraocular lens implantation (IOL) was

reported. Retinal structures were examined and DME and DR diagnosis were established according to the Early Treatment Diabetic Retinopathy Study Design (ETDRS) guidelines [14]. DME was considered in the following circumstances: retinal thickening or the presence of hard exudates within 1 disk diameter from the center of the fovea [15].The presence of microaneurysms, hemorrhages, exudates, venous beading, or neovascularization was taken into account in DR staging as follows: non-proliferative diabetic retinopathy (NPDR) with mild, moderate, and severe NPDR stages and proliferative diabetic retinopathy (PDR) [16]. IOP measurement was performed for all patients with a Perkins handheld applanation tonometer (Perkins Mk3 tonometer, Haag-Streit, Koniz, Switzerland). Patients' medical charts were also reported prior to the ophthalmologic treatments, including retinal laser photocoagulation or intravitreal injections with anti-VEGF.

2.3. Statistical Analysis

Statistical analysis was performed using the IBM Statistical Package for Social Sciences software (version 28, Armonk, NY, USA, accessed on 1 August 2024, https://www.ibm.com). The characteristics of the two study subgroups were presented using descriptive statistics, including the median, percentage, and interquartile range (IQR), depending on the variable type. The normality of the data distribution was evaluated using visual tools, such as histograms and probability plots, as well as analytical tests, specifically the Shapiro–Wilk test. Since the data were not normally distributed, numerical variables were reported as medians with the 25th and 75th IQRs, and the Mann–Whitney U test was used for comparisons between groups. Categorical variables were described as frequencies and percentages, with comparisons among groups performed using the Chi-squared or Fisher's exact test, depending on the data. The relationship between DME and the risk factors T1DM and T2DM was assessed using the point-biserial correlation (rpb) for continuous variables and the Phi coefficient (ϕ) for categorical variables. We also conducted a multivariate logistic regression to determine potential risk factors for DME within the study population. A *p*-value (two-tailed) of less than 0.05 was considered statistically significant.

3. Results

3.1. General Characteristics of the Study Population

The study included 105 participants, divided into two groups based on DME presence ($n = 77$) or absence ($n = 28$). The median age of the participants was 66 (IQR: 59–73) years. Those within the DME group were observed to be younger; however, this difference did not present a statistical significance value ($p = 0.071$). Both groups were similar regarding gender distribution, as seen in Table 1.

A total of 92.4% of the total study population had T2DM, and the rest T1DM. Disease duration was significantly longer in the DME group (median 15 years) compared to the group without DME (median 10 years), as seen in Figure 1. This indicates that patients with diabetes and DME had a longer DM duration than those without DME.

In terms of diabetes management, most of the patients were treated with non-insulin antidiabetic drugs, 30.5% were under treatment with insulin, and 12.4% received a combination of the two. There was no statistically significant difference in the distribution of these treatments between groups ($p = 0.219$). The current and maximum HbA1c levels were recorded, with the median values were slightly higher in the DME group (current HbA1c: 7.50%, IQR: 6.72–8.27; maximum HbA1c: 10.90%, IQR: 8.95–13) compared to the group without DME (current HbA1c: 6.85%, IQR: 6.42–7.80; maximum HbA1c: 9.45%, IQR: 8.85–10.70). The prevalence of dyslipidemia, HTN, obesity, and CVD comorbidities did not differ notably between the two studied groups. CKD was present only among patients who also presented with DME. Diabetic neuropathy was also more common in

patients with an associated diagnosis of DME, though this finding did not reach statistical significance. Still, all patients with DME had HTN in the researched population, 48.1% of them presenting moderate HTN, while dyslipidemia was diagnosed in 62.3% of the same subgroup. Furthermore, almost half of this subgroup presented with at least one other comorbidity (46.8%).

Table 1. General characteristics of the study population.

Variables	Total (n = 105)	DME (−) (n = 28)	DME (+) (n = 77)	p-Value
Age (years)	66 (59, 73)	69.5 (60.2, 74)	65 (58, 71.5)	0.071
Male n (%)	55 (52.4)	14 (50)	41 (53.2)	0.768
T1DM/T2DM n (%)	8 (7.6)/97 (92.4)	1 (3.6)/27 (96.4)	7 (9.1)/70 (90.9)	0.679
Disease duration	15 (8, 20)	10 (6.25, 15)	15 (9, 20)	**0.010**
Diabetes treatment n (%)				0.219
• OAD	58 (55.2)	19 (67.9)	39 (50.6)	
• INS	32 (30.5)	7 (25)	25 (32.5)	
• OAD + INS	13 (12.4)	1 (3.6)	12 (15.6)	
• OAD + weekly injection	2 (1.9)	1 (3.6)	1 (1.3)	
Current HbA1c %	7.30 (6.50, 8.07)	6.85 (6.42, 7.80)	7.50 (6.72, 8.27)	0.098
Maximum HbA1c %	10.1 (8.95, 12.55)	9.45 (8.85, 10.70)	10.90 (8.95, 13)	0.102
Dyslipidemia n (%)	70 (66.7)	22 (78.6)	48 (62.3)	0.161
HTN n (%)				0.292
• Mild	29.5 (31)	7 (25)	24 (31.2)	
• Moderate	48 (45.7)	11 (39.3)	37 (48.1)	
• Severe	26 (24.8)	10 (35.7)	16 (20.8)	
Obesity n (%)	31 (29.5)	10 (35.7)	21 (27.3)	0.402
Comorbidities n (%)	42 (40)	15 (53.6)	27 (35.1)	0.087
• CKD	11 (10.5)	0 (0)	11 (14.3)	**0.034**
• Diabetic neuropathy	13 (12.4)	1 (3.6)	12 (15.6)	0.177
• CVD	18 (17.1)	5 (17.9)	13 (16.9)	0.907
Smoking n (%)	22 (21)	3 (10.7)	19 (24.7)	0.120

Mann–Whitney U test and either the Chi-square (χ^2) test, Fisher's exact test, or Bowker's test, as appropriate. Data are expressed as the median, interquartile range (IQR), or percentage (n, %). DME, diabetic macular edema; DM, diabetes mellitus; OAD, oral antidiabetics; INS, insulin; HbA1c, hemoglobin A1c; HTN, hypertension; CKD, chronic kidney disease; CVD, cardiovascular disease. Differences that are statistically significant, defined as those with a value of $p < 0.05$, are highlighted in bold.

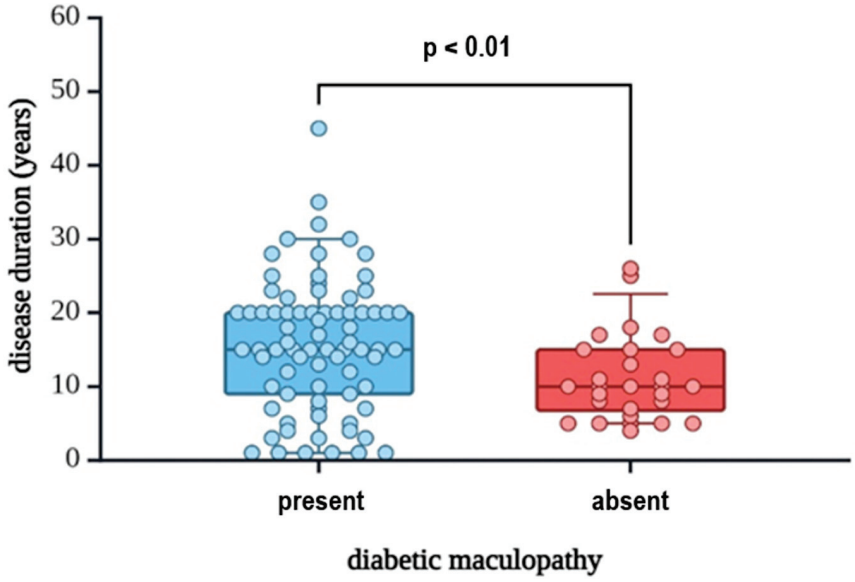

Figure 1. Disease duration and diabetic maculopathy status. Circles represent individual data points. Created with Biorender.com.

3.2. Ophthalmic Findings

Intraocular pressure (IOP) measurements in both the right eye (RE) and left eye (LE) did not vary significantly between groups. DR presence was significantly associated with DME ($p < 0.001$), with a greater proportion of patients in the DME group having PDR, as seen in Table 2. This led to the conclusion that macular edema was not only associated with diabetic retinopathy but also with more severe cases of the disease.

Table 2. Ophthalmologic characteristics of the study population.

Variables	Total (n = 105)	DME (−) (n = 28)	DME (+) (n = 77)	p-Value
IOP—RE mmHg	15 (14, 17)	16 (15, 17.7)	15 (13, 17)	0.204
IOP—LE mmHg	16 (14, 18)	16 (15, 18)	16 (13, 18)	0.266
Cataract n (%)				
• Absent	15 (14.3)	5 (17.9)	10 (12.9)	0.528
• Present	41 (39)	9 (32.1)	32 (41.6)	0.498
• Pseudophakia	49 (46.7)	14 (50)	35 (45.5)	0.515
Diabetic retinopathy n (%)				
• Absent	16 (15.2)	15 (53.5)	0	**<0.001**
• Mild non-proliferative	4 (3.8)	3 (10.7)	2 (2.6)	0.084
• Moderate non-proliferative	9 (8.6)	2 (7.2)	7 (9.1)	1
• Severe non-proliferative	34 (32.4)	6 (21.4)	28 (36.4)	0.166
• Proliferative	42 (40)	2 (7.2)	40 (51.9)	**<0.001**
Intravitreal injections n (%)	87 (82.9)	17 (60.7)	70 (90.9)	**<0.001**
Laser photocoagulation n (%)	56 (53.3)	6 (21.4)	50 (64.9)	**<0.001**

Mann–Whitney U test and either the Chi-square (χ^2) test or Fisher's exact test, as appropriate. Data are expressed as the median (interquartile range, IQR) or percentage (n, %). IOP, intraocular pressure; RE, right eye; LE, left eye; DME, diabetic macular edema. Statistically significant differences, with a $p < 0.05$, are highlighted in bold.

The use of intravitreal injections and laser photocoagulation was significantly more common in the DME group (both $p < 0.001$), indicating the need for more aggressive ophthalmologic intervention in these patients.

3.3. Correlation Analysis of DME with Risk Factors for T1DM and T2DM

Correlation analysis was conducted to assess which demographic or clinical factors correlated with the presence of DME (Table 3). A strong positive correlation was detected with DR ($r_{pb} = 0.643$), while weaker correlations were found with disease duration ($r_{pb} = 0.246$), current HbA1c ($\phi = 0.241$), and CKD ($r_{pb} = 0.206$).

Table 3. Correlation between DME and risk factors in T1DM and T2DM.

	Age	Gender	Disease Duration	Current HbA1c	Dys-Lipidemia	HTN	CKD	Diabetic Neuropathy	Smoking	DR
p-Value	0.070	0.768	**0.011**	**0.014**	0.119	0.117	**0.035**	0.098	0.273	**<0.001**
Correlation coefficient	−0.178	−0.029	0.246	0.241	0.152	−0.153	0.206	0.161	0.107	0.643

Point-biserial correlation (rpb) and Phi coefficient (ϕ), as appropriate. Abbreviations: DME, diabetic macular edema; HbA1c, hemoglobin A1c; HTN, hypertension; CKD, chronic kidney disease; Statistically significant differences, with a $p < 0.05$, are highlighted in bold.

3.4. Association Between DME and Several Demographic and Clinical Factors

In order to obtain more information regarding DME in patients with DM, a multivariate logistic regression analysis was performed, investigating potential factors associated with DME, as shown in Table 4. After adjusting for multiple confounding factors, including

risk factors T1DM and T2DM, only PDR remained statistically significant as an independent factor linked to DME occurrence ($p = 0.004$).

Table 4. Risk factors associated with DME in the study population.

Variables	Model 1		Model 2		Model 3	
	OR (95%CIs)	*p*-Value	OR (95%CIs)	*p*-Value	OR (95%CIs)	*p*-Value
Age	0.887 (0.438, 2.260)	0.802	0.922 (0.343, 2.476)	0.871	1.135 (0.388, 3.322)	0.818
Gender	0.948 (0.894, 1.005)	0.702	0.948 (0.890, 1.010)	0.970	0.954 (0.893, 1.020)	0.169
Disease duration	1.069 (1.005, 1.137)	**0.034**	1.088 (1.014, 1.166)	**0.018**	1.066 (0.990, 1.148)	0.089
Current HbA1C	1.312 (0.852, 2.021)	0.218	1.349 (0.843, 2.160)	0.212	1.088 (0.654, 1.809)	0.744
Severe HTN			0.471 (0.164, 1.351)	0.161	0.385 (0.119, 1.248)	0.112
Dyslipidemia			0.353 (0.113, 1.101)	0.073	0.500 (0.150, 1.674)	0.261
PDR					10.715 (2.16, 52.97)	**0.004**

Model 1 adjusted for: age; gender; disease duration; current HbA1c. Model 2 adjusted for: age; gender; disease duration; severe HTN; dyslipidemia. Model 3 adjusted for: age; gender; disease duration; severe HTN; dyslipidemia; PDR. OR, odds ratio; CI, confidence interval; HbA1c, hemoglobin A1c; HTN, hypertension; PDR, proliferative diabetic retinopathy. Differences that are statistically significant, defined as those with a probability value of $p < 0.05$, are highlighted in bold.

4. Discussion

The global prevalence of DME is increasing. As the number of DM cases rises, a corresponding rise in complications related to diabetes is expected [17]. With the worldwide increase in the aging population [18], the concept of multimorbidity has received significant attention among medical practitioners [19], particularly regarding patients with T2DM, who present a greater risk of having numerous co-occurring conditions [20]. Our research focused on observing DME cases in the western Romanian population with T2DM, which is known to be highly prevalent in this part of the country. Conditions such as arterial hypertension, chronic kidney disease, and obesity have a major impact on ocular complications related to DM, including vision-threatening DME [21].

In our study group, those who suffered from DME had longer DM duration, with a median of 15 years ($p = 0.010$), data that align with the current literature findings, which report a direct correlation between diabetes duration and the development and progression of ophthalmologic complications such as DME and DR. Notably, patients with T2DM might present DME at the time of DM diagnosis, underlining the importance of screening in both recently diagnosed patients, as well as patients who have been diagnosed for a longer period of time [6,22]. Regarding glycemic control, HbA1c levels were suboptimal in the whole research group but were particularly elevated among patients with DME (median value 7.5%, maximum value 10.9%). Increased HbA1c values are known to be linked to DME occurrence and progression, while conversely, adequate glycemic control positively impacts ocular-related complications. According to DDCT/EDIC, a 10–25% decrease in the risk of DME occurrence can be obtained by only a 1% decrease in HbA1c levels [23].

DR severity is the major risk factor linked to DME. Although DME can be found at any degree of DR, an increase in DR severity has been associated with the rising prevalence of DME [14]. Our research found a strong positive correlation between the presence of DR and DME ($p = 0.643$). Moreover, PDR was a statistically significant factor involved in DME occurrence ($p = 0.004$). Intravitreal injections and retinal laser photocoagulation prevailed as treatment strategies among our population, emphasizing the need for a more decisive approach when facing diabetes-related ocular complications. This situation seriously impacts DM patients' quality of life [24]. Even so, treatment outcomes do not always meet the desired results, suggesting other general health factors may have a greater impact on visual outcomes [25].

More than 60% of patients with DM present at least one comorbidity at the time of their DM diagnosis [26]. Our research population aligns with these findings, as the majority of the evaluated patients presented at least one comorbidity, arterial hypertension and dyslipidemia being the most prevalent pathologies among our DME subgroup. All these components lead to the appearance of metabolic syndrome, which in turn increases the risk of cardiovascular complications, one of the most serious ones being atrial fibrillation. Also, DME and metabolic syndrome are closely linked through their shared pathophysiological mechanisms, primarily related to insulin resistance, chronic inflammation, and vascular dysfunction [27]. All DME patients were diagnosed with HTN, with different degrees of severity, presenting a slight predominance towards moderate HTN (48.1%), according to data sustained by a study conducted by Zhang M et al. [28]. On the other hand, HTN presence, regardless of the severity, is linked to an increase in the probability of DME occurrence of 40% [29]. The Wisconsin Epidemiologic Study of Diabetic Retinopathy found that systemic hypertension triples the prevalence of DME. It has been proposed that hypertension serves as a risk factor for the development of macular edema, and its management could provide significant benefits for patients with uncontrolled blood pressure. Sustaining the importance of HTN management, according to UKPDS 69, a reduction of only 10 mmHg in systolic blood pressure reduced the risk of DME development by 15% [30], while the optimal blood pressure values assessment among patients with DM could be represented by a 24 h measurement, due to the more significantly predictive power concerning the evaluation cardiovascular risk in these patients [31].

Previous studies have indicated that dyslipidemia is linked to DME. Furthermore, a global multicenter study found that high triglyceride levels and low HDL cholesterol levels were associated with an increased risk of diabetic microvascular diseases. Dyslipidemia is one of the most frequent comorbidities found in patients with DM [26]. Our research revealed that 66.7% of evaluated patients presented this pathology, 62.3% belonging to the DME subgroup. Treating dyslipidemia should address all cholesterol fractions, yet elevating HDL-cholesterol levels must become a therapeutic target, especially among patients with DME, as it was found to decrease the risk of DME, as stated by Klein et al. and confirmed by another study conducted by Chung et al. [32,33].

Besides HTN and dyslipidemia, 46.8% of the DME subpopulation presented at least one other comorbidity, such as CKD, diabetic polyneuropathy, or CVD. Regarding these comorbidities, we found a statistically significant correlation between CKD and DME ($p = 0.034$). Multiple reports have evaluated the relationship between DME and CKD. Acan et al. reported CKD is a risk factor for DME development [13], while another study from Melbourne observed no associations between GFR and DME in 61 Caucasian patients with DME [34]. In contrast, another population-based study indicated that CKD was significantly associated with incident DME during a 10-year follow-up examination [35]. The positive association between DME and GFR was also sustained by a single-center retrospective observational study involving patients with T2DM in South China [36]. These different results among various studies suggest that although both CKD and DME share a comparable pathology mechanism, microangiopathy, the exact influence of renal factors on DME remains unclear [37].

Lifestyle-related habits are also of great importance when deciding upon a therapeutic approach for a patient with DM and DME [38]. Obesity is known to be especially prevalent in patients with T2DM, and its management becomes mandatory, as lowering body mass is known to have a beneficial effect on visual outcomes post-DME treatment [39]. In our research, 27.3% of DME patients had obesity, results that the relatively small research group might determine. We consider that further research is necessary in this direction, as there

are studies that link not only BMI values but also abdominal fat distribution (waist-to-hip ratio) to the development of diabetes-related ocular pathologies [39,40].

Regarding smoking addiction, we have found that 24.7% of patients with DME were active smokers. Since smoking was self-reported, we did not take past or occasional smokers into account, as we considered it would impede result precision. However, we underscore the need for these categories to be included in future research. Data regarding the direct impact of smoking on DME is somewhat inconclusive in the existing literature. Still, smoking cessation should be advised in all patients with DM, as nicotine is known to impair insulin activity and negatively impact glycemic control [41,42].

As shown in existing studies and as we have observed in the present study, most of the patients with DME have multiple associated diseases that facilitate the appearance of macular edema and accelerate its progression. For this reason, it is very important to identify these pathologies as early as possible so that through their correct management, we can try to prevent the occurrence and subsequent progression of DME.

Although somewhat restricted by the relatively small population size, this research accurately reflects the general health status among patients with DM with DME evaluated in hospital settings in the western part of our country, and the baseline health characteristics of the studied cohort are reflected with precision. Furthermore, considering the consecutive patient enrollment in this research, the heterogeneity of the final study group emphasizes the diversity of patients that medical practitioners encounter in daily clinical practice, accentuating the need for a personalized medical approach for each patient.

Our results have shown that patients with DME generally present multiple associated comorbidities. Therefore, a stringent treatment plan is mandatory, which is an objective achieved in Romania for DM patients by regular check-ups every 3 months, while chronic complication screening is usually performed annually. These particular visits also address patient education regarding diabetes and diabetes treatment. Yet, further education is needed concerning the associated general and ocular diseases and their symptoms to better understand the disease and facilitate better treatment adherence among patients. Besides an increase in the degree of health education, as multiple comorbidities threaten each diabetic patient's quality of life, we consider that general and ophthalmologic health status screening should be performed at least every 6 months, as a proper diagnosis timing could lead to the more personalized and effective management of these patients.

Regarding the ophthalmologic evaluation of diabetic patients, while medical settings equipped with optical coherence tomography (OCT) would represent the ideal scenario since it represents the gold standard in the diagnosis of DME and other diabetes-related pathologies, the patients often do not have access to hospitals equipped as such. As such, it is important that screening is performed for all patients with DM, whether at the initial consultation or well into their diagnosis [43]. Still, we emphasize the importance of an extended ophthalmologic examination using fundus photography and OCT for a personalized treatment protocol and a precise follow-up.

Future research perspectives also refer to expanding the study population to a larger and younger demographic group while conducting a multicenter, prospective study for a more accurate representation of diabetes-related ocular complications such as DME and associated comorbidities and their interrelation, the main objective remaining individualized patient management and the subsequent quality of life improvement.

5. Conclusions

This body of research underlines the importance of close monitoring for DME in patients with DM, underlining the risks represented by this complication and the major impact on the quality of life of these patients while simultaneously identifying individuals

for which DME is more prevalent. In all evaluated patients associated with at least one other comorbidity, HTN, dyslipidemia, or CKD were more prevalent and are known to be the most significant risk factors involved in DME occurrence.

Considering the continuously growing numbers of people with DM worldwide, the necessity of personalized medicine becomes more and more evident, especially given the multitude of complications that accompany this disease. A personalized approach could translate into early complication detection, better quality of life, and improved morbidity and mortality rates. In this particular case, patients with DM would benefit from early DME detection and subsequent management, guaranteeing sight preservation.

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Informed Consent Statement: Informed consent was obtained from all participants involved in the present study.

Data Availability Statement: All available data can be provided upon request to the corresponding author.

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References

1. Sun, H.; Saeedi, P.; Karuranga, S.; Pinkepank, M.; Ogurtsova, K.; Duncan, B.B.; Stein, C.; Basit, A.; Chan, J.C.N.; Mbanya, J.C.; et al. IDF Diabetes Atlas: Global, Regional and Country-Level Diabetes Prevalence Estimates for 2021 and Projections for 2045. *Diabetes Res. Clin. Pract.* **2022**, *183*, 109119. [CrossRef] [PubMed]
2. Zhang, J.; Zhang, J.; Zhang, C.; Zhang, J.; Gu, L.; Luo, D.; Qiu, Q. Diabetic Macular Edema: Current Understanding, Molecular Mechanisms and Therapeutic Implications. *Cells* **2022**, *11*, 3362. [CrossRef] [PubMed]
3. Mrugacz, M.; Pony-Uram, M.; Bryl, A.; Zorena, K. Current Approach to the Pathogenesis of Diabetic Cataracts. *Int. J. Mol. Sci.* **2023**, *24*, 6317. [CrossRef]
4. Shah, J.; Nguyen, V.; Hunt, A.; Mehta, H.; Romero-Nuñez, B.; Zarranz-Ventura, J.; Viola, F.; Bougamha, W.; Barnes, R.; Barthelmes, D.; et al. Characterization of Poor Visual Outcomes of Diabetic Macular Edema: The Fight Retinal Blindness! Project. *Ophthalmol. Retin.* **2022**, *6*, 540–547. [CrossRef]
5. Chew, E.Y.; Davis, M.D.; Danis, R.P.; Lovato, J.F.; Perdue, L.H.; Greven, C.; Genuth, S.; Goff, D.C.; Leiter, L.A.; Ismail-Beigi, F.; et al. The Effects of Medical Management on the Progression of Diabetic Retinopathy in Persons with Type 2 Diabetes. *Ophthalmology* **2014**, *121*, 2443–2451. [CrossRef]
6. Musat, O.; Cernat, C.; Labib, M.; Gheorghe, A.; Toma, O.; Zamfir, M.; Boureanu, A.M. Diabetic Macular Edema. *Rom. J. Ophthalmol.* **2015**, *59*, 133–136. [PubMed]
7. Thomas, R.L.; Dunstan, F.D.; Luzio, S.D.; Chowdhury, S.R.; North, R.V.; Hale, S.L.; Gibbins, R.L.; Owens, D.R. Prevalence of Diabetic Retinopathy within a National Diabetic Retinopathy Screening Service. *Br. J. Ophthalmol.* **2015**, *99*, 64–68. [CrossRef]
8. Yau, J.W.Y.; Rogers, S.L.; Kawasaki, R.; Lamoureux, E.L.; Kowalski, J.W.; Bek, T.; Chen, S.-J.; Dekker, J.M.; Fletcher, A.; Grauslund, J.; et al. Global Prevalence and Major Risk Factors of Diabetic Retinopathy. *Diabetes Care* **2012**, *35*, 556–564. [CrossRef] [PubMed]
9. Yu, A.J.; Masalkhi, M.; Brown, R.; Chen, B.; Chhablani, J. Racial and Ethnic Distribution in Diabetic Macular Edema Clinical Trials in the United States (2002–2021). *Ophthalmol. Retin.* **2023**, *7*, 1035–1041. [CrossRef]

10. Braha, A.; Simion, A.; Timar, R.; Timar, B. Factors Associated with Increased Intraocular Pressure in Type 2 Diabetes Patients. *J. Clin. Med.* **2024**, *13*, 676. [CrossRef]
11. Busch, C.; Katzmann, J.L.; Jochmann, C.; Unterlauft, J.D.; Vollhardt, D.; Wiedemann, P.; Laufs, U.; Rehak, M. General Health of Patients with Diabetic Macular Edema—The LIPSIA Study. *PLoS ONE* **2021**, *16*, e0252321. [CrossRef] [PubMed]
12. Eleftheriadou, A.; Riley, D.; Zhao, S.S.; Austin, P.; Hernández, G.; Lip, G.Y.H.; Jackson, T.L.; Wilding, J.P.H.; Alam, U. Risk of Diabetic Retinopathy and Diabetic Macular Oedema with Sodium–Glucose Cotransporter 2 Inhibitors and Glucagon-like Peptide 1 Receptor Agonists in Type 2 Diabetes: A Real-World Data Study from a Global Federated Database. *Diabetologia* **2024**, *67*, 1271–1282. [CrossRef] [PubMed]
13. May, M.; Framke, T.; Junker, B.; Framme, C.; Pielen, A.; Schindler, C. How and Why SGLT2 Inhibitors Should Be Explored as Potential Treatment Option in Diabetic Retinopathy: Clinical Concept and Methodology. *Ther. Adv. Endocrinol.* **2019**, *10*, 204201881989188. [CrossRef]
14. Early Treatment Diabetic Retinopathy Study Design and Baseline Patient Characteristics. *Ophthalmology* **1991**, *98*, 741–756. [CrossRef] [PubMed]
15. Gundogan, F.C.; Yolcu, U.; Akay, F.; Ilhan, A.; Ozge, G.; Uzun, S. Diabetic Macular Edema: A Short Review. *Pak. J. Med. Sci.* **2016**, *32*, 505–510. [CrossRef]
16. Yang, Z.; Tan, T.-E.; Shao, Y.; Wong, T.Y.; Li, X. Classification of Diabetic Retinopathy: Past, Present and Future. *Front. Endocrinol.* **2022**, *13*, 1079217. [CrossRef] [PubMed]
17. Acan, D.; Calan, M.; Er, D.; Arkan, T.; Kocak, N.; Bayraktar, F.; Kaynak, S. The Prevalence and Systemic Risk Factors of Diabetic Macular Edema: A Cross-Sectional Study from Turkey. *BMC Ophthalmol.* **2018**, *18*, 91. [CrossRef]
18. Shehan, C.L. (Ed.) *The Wiley Blackwell Encyclopedia of Family Studies*; John Wiley & Sons, Inc.: Chichester, West Sussex, UK, 2016; ISBN 978-1-119-08562-1.
19. Nicholson, K.; Almirall, J.; Fortin, M. The Measurement of Multimorbidity. *Health Psychol.* **2019**, *38*, 783–790. [CrossRef]
20. Cicek, M.; Buckley, J.; Pearson-Stuttard, J.; Gregg, E.W. Characterizing Multimorbidity from Type 2 Diabetes. *Endocrinol. Metab. Clin. N. Am.* **2021**, *50*, 531–558. [CrossRef]
21. Lee, R.; Wong, T.Y.; Sabanayagam, C. Epidemiology of Diabetic Retinopathy, Diabetic Macular Edema and Related Vision Loss. *Eye Vis.* **2015**, *2*, 17. [CrossRef]
22. Klein, R.; Knudtson, M.D.; Lee, K.E.; Gangnon, R.; Klein, B.E.K. The Wisconsin Epidemiologic Study of Diabetic Retinopathy XXII. *Ophthalmology* **2008**, *115*, 1859–1868. [CrossRef] [PubMed]
23. Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Study Research Group Intensive Diabetes Treatment and Cardiovascular Outcomes in Type 1 Diabetes: The DCCT/EDIC Study 30-Year Follow-Up. *Diabetes Care* **2016**, *39*, 686–693. [CrossRef]
24. Fernández-Vigo, J.I.; Contreras, I.; Crespo, M.J.; Beckford, C.; Flores-Moreno, I.; Cobo-Soriano, R.; Pareja, J.; Martín, M.D.; Moreno, L.; Arrevola-Velasco, L. Expert Panel Consensus for Addressing Anti-VEGF Treatment Challenges of Diabetic Macular Edema in Spain. *OPTH* **2022**, *16*, 3097–3106. [CrossRef]
25. Gurung, R.L.; FitzGerald, L.M.; Liu, E.; McComish, B.J.; Kaidonis, G.; Ridge, B.; Hewitt, A.W.; Vote, B.J.; Verma, N.; Craig, J.E.; et al. Predictive Factors for Treatment Outcomes with Intravitreal Anti-Vascular Endothelial Growth Factor Injections in Diabetic Macular Edema in Clinical Practice. *Int. J. Retin. Vitr.* **2023**, *9*, 23. [CrossRef]
26. Iglay, K.; Hannachi, H.; Joseph Howie, P.; Xu, J.; Li, X.; Engel, S.S.; Moore, L.M.; Rajpathak, S. Prevalence and Co-Prevalence of Comorbidities among Patients with Type 2 Diabetes Mellitus. *Curr. Med. Res. Opin.* **2016**, *32*, 1243–1252. [CrossRef] [PubMed]
27. Rosca, C.I.; Lighezan, D.F.; Nisulescu, D.-D.; Sharma, A.; Neagu, M.N.; Nistor, D.; Georgescu, D.; Kundnani, N.R. Metabolic Syndrome: A Strange Companion of Atrial Fibrillation; A Blessing in Disguise from the Neuropsychiatric Point of View. *Biomedicines* **2023**, *11*, 2012. [CrossRef]
28. Zhang, M.; Wu, J.; Wang, Y.; Wu, J.; Hu, W.; Jia, H.; Sun, X. Associations between Blood Pressure Levels and Diabetic Retinopathy in Patients with Diabetes Mellitus: A Population-Based Study. *Heliyon* **2023**, *9*, e16830. [CrossRef] [PubMed]
29. Klein, R.; Klein, B.E.; Moss, S.E.; Cruickshanks, K.J. The Wisconsin Epidemiologic Study of Diabetic Retinopathy: XVII. The 14-Year Incidence and Progression of Diabetic Retinopathy and Associated Risk Factors in Type 1 Diabetes. *Ophthalmology* **1998**, *105*, 1801–1815. [CrossRef]
30. Matthews, D.R.; Stratton, I.M.; Aldington, S.J.; Holman, R.R.; Kohner, E.M.; UK Prospective Diabetes Study Group. Risks of Progression of Retinopathy and Vision Loss Related to Tight Blood Pressure Control in Type 2 Diabetes Mellitus: UKPDS 69. *Arch. Ophthalmol.* **2004**, *122*, 1631–1640. [CrossRef]
31. O'Brien, E.; Parati, G.; Stergiou, G.; Asmar, R.; Beilin, L.; Bilo, G.; Clement, D.; de la Sierra, A.; de Leeuw, P.; Dolan, E.; et al. European Society of Hypertension Position Paper on Ambulatory Blood Pressure Monitoring. *J. Hypertens.* **2013**, *31*, 1731–1768. [CrossRef]

32. Klein, B.E.K.; Myers, C.E.; Howard, K.P.; Klein, R. Serum Lipids and Proliferative Diabetic Retinopathy and Macular Edema in Persons With Long-Term Type 1 Diabetes Mellitus: The Wisconsin Epidemiologic Study of Diabetic Retinopathy. *JAMA Ophthalmol.* **2015**, *133*, 503–510. [CrossRef] [PubMed]
33. Chung, Y.-R.; Park, S.W.; Choi, S.-Y.; Kim, S.W.; Moon, K.Y.; Kim, J.H.; Lee, K. Association of Statin Use and Hypertriglyceridemia with Diabetic Macular Edema in Patients with Type 2 Diabetes and Diabetic Retinopathy. *Cardiovasc. Diabetol.* **2017**, *16*, 4. [CrossRef] [PubMed]
34. Man, R.E.K.; Sasongko, M.B.; Wang, J.J.; MacIsaac, R.; Wong, T.Y.; Sabanayagam, C.; Lamoureux, E.L. The Association of Estimated Glomerular Filtration Rate With Diabetic Retinopathy and Macular Edema. *Investig. Ophthalmol. Vis. Sci.* **2015**, *56*, 4810. [CrossRef] [PubMed]
35. Romero-Aroca, P.; Baget-Bernaldiz, M.; Navarro-Gil, R.; Moreno-Ribas, A.; Valls-Mateu, A.; Sagarra-Alamo, R.; Barrot-De La Puente, J.F.; Mundet-Tuduri, X. Glomerular Filtration Rate and/or Ratio of Urine Albumin to Creatinine as Markers for Diabetic Retinopathy: A Ten-Year Follow-Up Study. *J. Diabetes Res.* **2018**, *2018*, 5637130. [CrossRef]
36. Zhuang, X.; Cao, D.; Yang, D.; Zeng, Y.; Yu, H.; Wang, J.; Kuang, J.; Xie, J.; Zhang, S.; Zhang, L. Association of Diabetic Retinopathy and Diabetic Macular Oedema with Renal Function in Southern Chinese Patients with Type 2 Diabetes Mellitus: A Single-Centre Observational Study. *BMJ Open* **2019**, *9*, e031194. [CrossRef]
37. Wang, L.; Jin, L.; Wang, W.; Gong, X.; Li, Y.; Li, W.; Liang, X.; Huang, W.; Liu, Y. Association of Renal Function with Diabetic Retinopathy and Macular Oedema among Chinese Patients with Type 2 Diabetes Mellitus. *Eye* **2023**, *37*, 1538–1544. [CrossRef]
38. Bhattacharjee, H.; Barman, M.; Garg, M. Diabetic Retinopathy and Diabetic Macular Edema: Fighting the Emerging Global Burden. In *Diabetic Macular Edema*; Saxena, S., Cheung, G., Lai, T.Y.Y., Sadda, S.R., Eds.; Springer Nature: Singapore, 2022; pp. 221–227. ISBN 978-981-19730-6-2.
39. Graue-Hernandez, E.O.; Rivera-De-La-Parra, D.; Hernandez-Jimenez, S.; Aguilar-Salinas, C.A.; Kershenobich-Stalnikowitz, D.; Jimenez-Corona, A. Prevalence and Associated Risk Factors of Diabetic Retinopathy and Macular Oedema in Patients Recently Diagnosed with Type 2 Diabetes. *BMJ Open Ophthalmol.* **2020**, *5*, e000304. [CrossRef]
40. Hwang, I.C.; Bae, J.H.; Kim, J.M. Relationship between Body Fat and Diabetic Retinopathy in Patients with Type 2 Diabetes: A Nationwide Survey in Korea. *Eye* **2019**, *33*, 980–987. [CrossRef]
41. Boretsky, A.; Gupta, P.; Tirgan, N.; Liu, R.; Godley, B.F.; Zhang, W.; Tilton, R.G.; Motamedi, M. Nicotine Accelerates Diabetes-Induced Retinal Changes. *Curr. Eye Res.* **2015**, *40*, 368–377. [CrossRef]
42. Cai, X.; Chen, Y.; Yang, W.; Gao, X.; Han, X.; Ji, L. The Association of Smoking and Risk of Diabetic Retinopathy in Patients with Type 1 and Type 2 Diabetes: A Meta-Analysis. *Endocrine* **2018**, *62*, 299–306. [CrossRef]
43. Nicoară, S.D. Spectral Domain Optical Coherence Tomography in the Diagnosis and Monitoring of Diabetic Macular Edema. In *OCT—Applications in Ophthalmology*; Lanza, M., Ed.; InTech: London, UK, 2018; ISBN 978-1-78923-754-2.

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Corrected Axial Length and Choroidal Thickness: A Correlation Analysis for Scientific Purposes

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Abstract: Purpose: Choroidal thickness (ChT) is an important measurement for evaluating eye and systemic disorders, but it is influenced by numerous elements, especially axial length (AL). It is known that the presence of a linear relationship between ChT and AL exists, but recently it has been shown that the AL measurement obtained with the current optical biometry is not very precise and needs to be corrected. This study aimed to verify if a similar correlation also persists with this corrected AL (ALc). **Methods:** All subjects underwent a complete eye examination, including spectral domain optical coherence tomography (OCT) with enhanced depth image (EDI) mode and AL measurement with IOLMaster. After a normality check of the data, the correlations between ChT with AL and ALc were investigated through the Pearson correlation coefficient. p values < 0.05 were considered statistically significant. **Results:** In total, 100 eyes of 50 healthy patients were evaluated. The mean AL was 24.36 ± 1.23 mm and mean ALc was 24.25 ± 1.22 mm. The mean nasal ChT, subfoveal ChT, and temporal ChT were, respectively, 250.57 ± 93.93 μm, 307.18 ± 101.66 μm, and 313.72 ± 88.86 μm. A significant negative linear correlation was found by comparing both AL and ALc to ChT (all r < −0.500, all p < 0.050). The negative linear correlation was stronger between nasal ChT and both AL and ALc (all r = −0.581). **Conclusions:** Through OCT and optical biometry, we confirmed that a statistically significant correlation persists between ALc and ChT, equal to the uncorrected AL. On these bases, in ChT studies or protocols, we recommend stratifying population according to ALc because linear correlation is still present; however, the cut-off values should be changed according to the systematic errors in optical biometry. In addition, both AL and ChT changes should be evaluated according to ALc.

Keywords: axial length; choroidal thickness; corrected axial length; ChT-AL correlation

1. Introduction

Choroid evaluation is becoming an emerging topic in ophthalmology due to its role in the diagnosis and follow-up of several pathologies and conditions [1–3]. Spaide et al., with their pioneering studies, first described a method of obtaining images of the choroid using conventional optical coherence tomography (OCT) with the aim of evaluating choroidal thickness (ChT) measurements using these images [4–6]. In fact, in the last few years, several authors investigated the role of ChT in several systemic and eye diseases, even though the assessment of this choroidal parameter is very difficult because it is affected by several factors, such as body position, axial length (AL), drug intake, and drug withdrawal [1,2]. The relationship between AL and ChT has been studied in detail: in fact, it is known in

the scientific literature that a significant relationship exists between AL and ChT [2,3,7–17]. A linear relationship seems to be present between AL and ChT, with short eyes having a thicker choroid, and long eyes having a thinner one [3].

For this reason, in a study evaluating ChT and ChT changes, it essential to stratify subjects according to AL, in order to obtain homogeneous and comparable samples. However, studies that evaluate ChT generally do not consider that AL evaluation, especially in patients affected by cataracts, are more challenging and the risk of bias is really high. In fact, especially in cases of biometry being based on group refractive index (GRI) or when evaluating extremely long eyes, AL measurements need to be corrected [18].

Several authors proposed different AL correcting factors [18–22], but most of them are strictly designed for IOL power calculation [19–22]. Instead, other correcting equations are based on erroneous AL measurements due to lens opacity or GRI-based biometry [18,22], such as the corrected AL (ALc) by De Bernardo et al. [18].

Corrected ALs potentially have a double impact in ChT evaluation studies:

(1) A change in the relationship between AL and ChT measurements could occur;
(2) More important, the choice of AL cut-off values in these types of studies could change accordingly.

Regarding point 1, if we consider that many AL correction factors are generally applied only in specific AL ranges [19–22], they could influence the linear relationship with a "zone of conflict" when passing the cut-off values for adjustment application. For example, Wang–Koch adjustments, which are usually applied in case of long eyes, could modify this linear relationship.

Regarding point 2, the cut-off values of AL in ChT studies become crucial: the choice of the right values is essential for patients' recruitment, samples stratifications, change analysis, and outcome evaluations. To better understand the impact of corrected AL on ChT studies, some examples of AL/ChT studies are reported:

- AL changes related to ChT changes in pharmacological studies [23];
- AL and ChT changes in myopia studies in children and adolescents [24];
- AL and ChT changes in myopia studies in adults [25];
- AL and ChT changes in accommodation studies [14];
- AL stratification in ChT studies [26].

Why do we pay great attention to ChT reliability, and also forget to take AL reliability in choroidal studies into consideration?

We tried to answer this question and so far, to the best of our knowledge, no studies investigated the influence of corrected AL with ChT. Does the relationship between AL and ChT remain unaltered? If not, how should studies evaluating ChT stratify samples? The purpose of this study was to evaluate the correlation between ChT and ALc, because only this latter AL adjustment is not exclusively linked to IOL power calculation accuracy; however, it eliminates systematic error in biometry.

2. Materials and Methods

2.1. Participants

In this study, healthy subjects with no ocular diseases which could influence the AL or OCT measurement, such as cataract or corneal and vitreous opacities, were recruited between September 2022 and January 2023. The exclusion criteria were systemic and ocular diseases, previous eye surgery, age under 18 or over 40 years old, pregnant women and eyes with AL < 21.00 mm or AL > 27.50 mm.

The present study adhered to the ethical principles of the Declaration of Helsinki. All the participants were carefully informed about the purpose of the study and written informed consent was acquired from each subject. Institutional Review Board (IRB) approval was also obtained from the ComEtico Campania Sud (CECS), prot. n°16544.2.2

2.2. Clinical and Instrumental Examination

All patients underwent a complete eye examination consisting of the following:

- slit lamp inspection;
- visual acuity test;
- fundus examination.

All recruited patients had a best corrected visual acuity >20/25 and absence of abnormalities at both slit lamp and fundus examinations. The evaluation also included a spectral domain optical coherence tomography (OCT) evaluation with enhanced depth image (EDI) mode (Spectralis; Heidelberg Engineering; Heidelberg, Germany, version 6.0) and AL measurement with IOLMaster (Carl Zeiss Meditec AG, Jena, Germany, version 5.4.4.0006). The OCT exams were performed without the instillation of mydriatics eye drops, given the related ChT thinning in the temporal sector [23].

2.2.1. OCT Analysis

OCT scans were performed with the Heidelberg Spectralis spectral domain (SD) device. The SD-OCT uses a spectrometer to detect the interference pattern of the light reflected from different tissue layers [27]. SD-OCTs can produce high-speed images of the tissue with high axial resolution, for this reason, they are widely used in ophthalmology to perform the diagnosis and management of several pathologies [27]. The OCT analysis was performed using the OCT software measurement tool (Heidelberg Eye Explorer HEYEX, version 5.3; Heidelberg Engineering). The macular region was evaluated through a horizontal 30° linear OCT B-scan passing through the fovea, with an average of 100 frames for every B-scan. Only scans through the fovea and images with a high signal-to-noise ratio (minimum of 20 dB) were included and used for the ChT evaluation. All subjects were analyzed between 2:00 p.m. and 4:00 p.m. in order to reduce bias related to the diurnal ChT variations.

The ChT assessment was performed at the subfoveal level, at 1500 μm nasally and temporally from the fovea, perpendicularly from the hyper-reflective line of the outer border of the retinal pigment epithelium to the hyper-reflective sclero-choroidal junction centered at the base of the fovea.

2.2.2. AL Measurements

The AL measurements were performed in the routinely used method with an IOL Master 500, which is a GRI-based biometer; it measures AL utilizing partial coherence interferometry (PCI) technology. AL was measured with a semiconductor laser diode that emits a dual beam of infrared light (780 nm). The signal produced by the interference between the light reflected from the tear film and the one reflected by the retinal pigment epithelium is received by the photodetector, which is used to calculate the optical distance between the corneal surface and the retina. This optical distance is used to calculate the geometrical intraocular distances [28]. In addition, IOLMaster 500 measures keratometry values at a 2.5 mm zone on the anterior cornea with six measured points, and it measures anterior chamber depth (ACD) using lateral slit illumination [28]. The AL measurements were taken utilizing the phakic option available on the biometer. The AL values were then corrected utilizing the following formula from De Bernardo et al.:

$$ALc = -0.017 + 0.996 * AL$$

where ALc is the corrected AL, and AL is the AL detected with the optical biometer by utilizing the phakic option [18]. Contrary to many other AL adjustments, ALc should be used independently from a specific AL range, because it is based on the assumption of a systematic error correction in the optical biometer, noting the influence of lens opacity and GRI on the reliability of the AL measurement. For this reason, the AL of all participants was corrected [29].

2.3. Statistical Analysis

Data collection was performed with Microsoft Excel and statistical analysis with IBM SPSS software (International Business Machine Corporation, Armonk, NY, USA, version 26.0). The Kolmogorov–Smirnov Test was performed to assess the normal distribution of the data. The Student T-test was used for pair-wise comparisons of AL and ALc. The correlations between ChT with AL and ALc were investigated using the Pearson correlation coefficient (r) and the Pearson coefficient of determination. Pearson's analysis with bootstrap was also performed in additional evaluation according to eye-side subgroups. In all analyses, p values < 0.05 were considered statistically significant. Additionally, the mean, standard deviation, median, minimum, and maximum were calculated for each data point.

The preliminary sample size was calculated with G*Power software (Version 3.1.9.7, Faul, Erdfelder, Lang, & Buchner, 2020.Available at https://www.gpower.hhu.de, access date: 10 October 2024). Given a conventional effect size q of 0.6, it was estimated that a sample size of 47 eyes for each group (94 eyes in total) would be necessary, with a significance level of 5% and a test power of 80%.

3. Results

In total, 100 eyes of 50 healthy subjects (23 males and 22 females) with a mean age of 27.6 ± 2.3 years (ranging from 24 to 35 years) were evaluated. Both AL measurements and ChT data were normally distributed (all $p > 0.050$). The descriptive statistics of the data are reported in Table 1.

Table 1. Descriptive statistics of uncorrected and corrected axial length and nasal, subfoveal, and temporal choroidal thickness.

Parameter	AL	ALc	ChT Nasal	ChT Subfoveal	ChT Temporal
MEAN	24.36 mm	24.25 mm	250.57 µm	307.18 µm	313.72 µm
SD	1.23 mm	1.22 mm	93.03 µm	101.66 µm	88.86 µm
MEDIAN	24.12 mm	24.00 mm	250.00 µm	299.50 µm	301.00 µm
MIN	21.49 mm	21.39 mm	73.00 µm	75.00 µm	135.00 µm
MAX	27.31 mm	27.18 mm	507.00 µm	504.00 µm	501.00 µm

SD: standard deviation; Min: minimum; Max: maximum; AL: axial length, ALc: corrected axial length according to De Bernardo et al.; ChT: choroidal thickness.

A statistically significant decrease was found with ALc compared to AL: mean difference was −0.11 mm ± 0.06 mm ($p < 0.001$).

Even if a small change in slopes and intercepts in linear relationships analysis was found, as reported in Figures 1–3, a moderate negative correlation persists between both AL and ALc and ChT in different sectors. In detail, the findings were as follows:

- a negative correlation was found between both AL and ALc with subfoveal ChT (r: −0.581, $p < 0.001$);
- a negative correlation was found between both AL and ALc with nasal ChT (r: −0.527, $p < 0.001$);

- a negative correlation was found between both AL and ALc with temporal ChT (r: −0.577, $p < 0.001$).

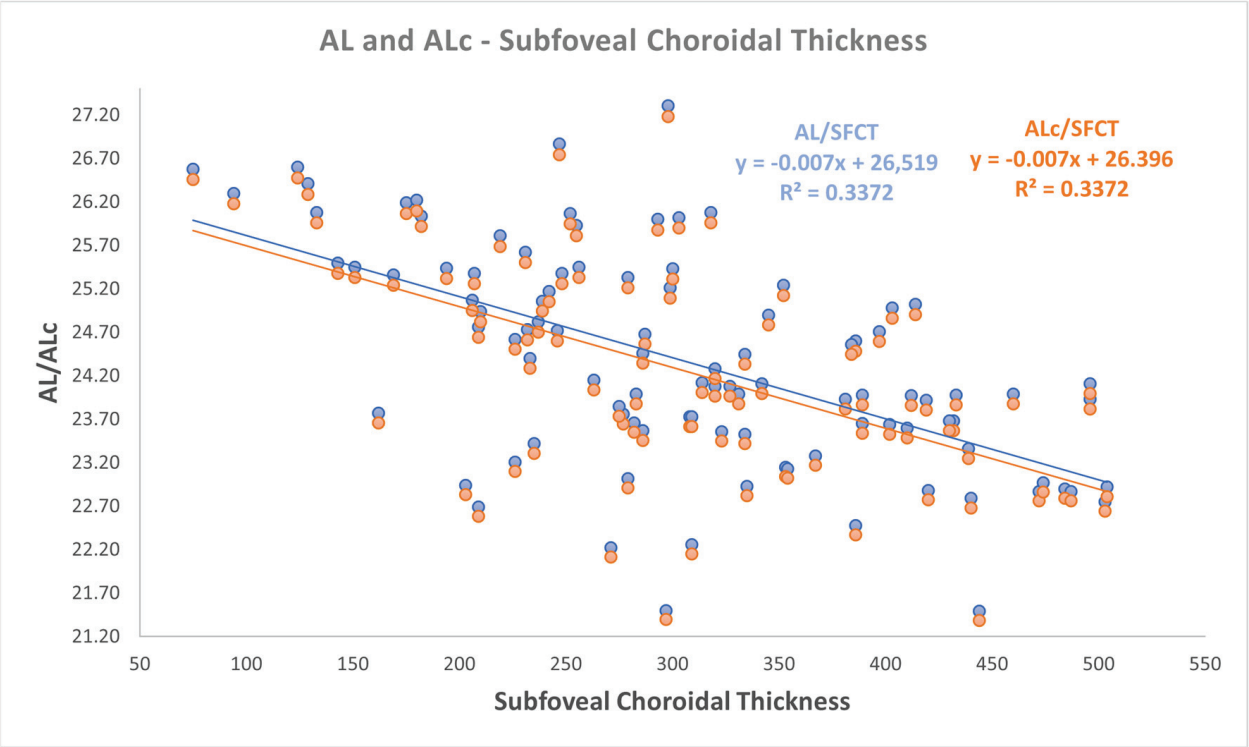

Figure 1. The relationship between both axial length (AL) and corrected AL (ALc) with subfoveal choroidal thickness.

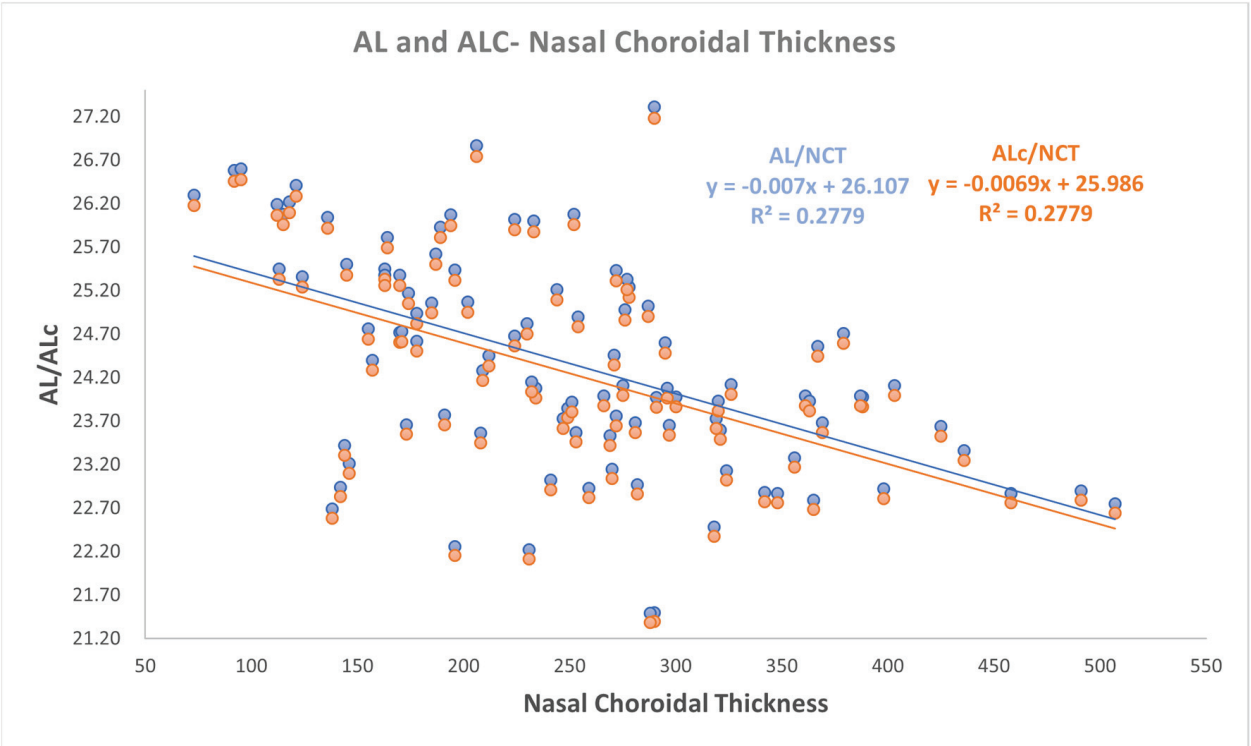

Figure 2. The relationship between both axial length (AL) and corrected AL (ALc) with nasal choroidal thickness.

Correlation analyses between AL and the choroidal parameters also with coefficient of determination according to Pearson are defined in Table 2 and in Figures 1–3.

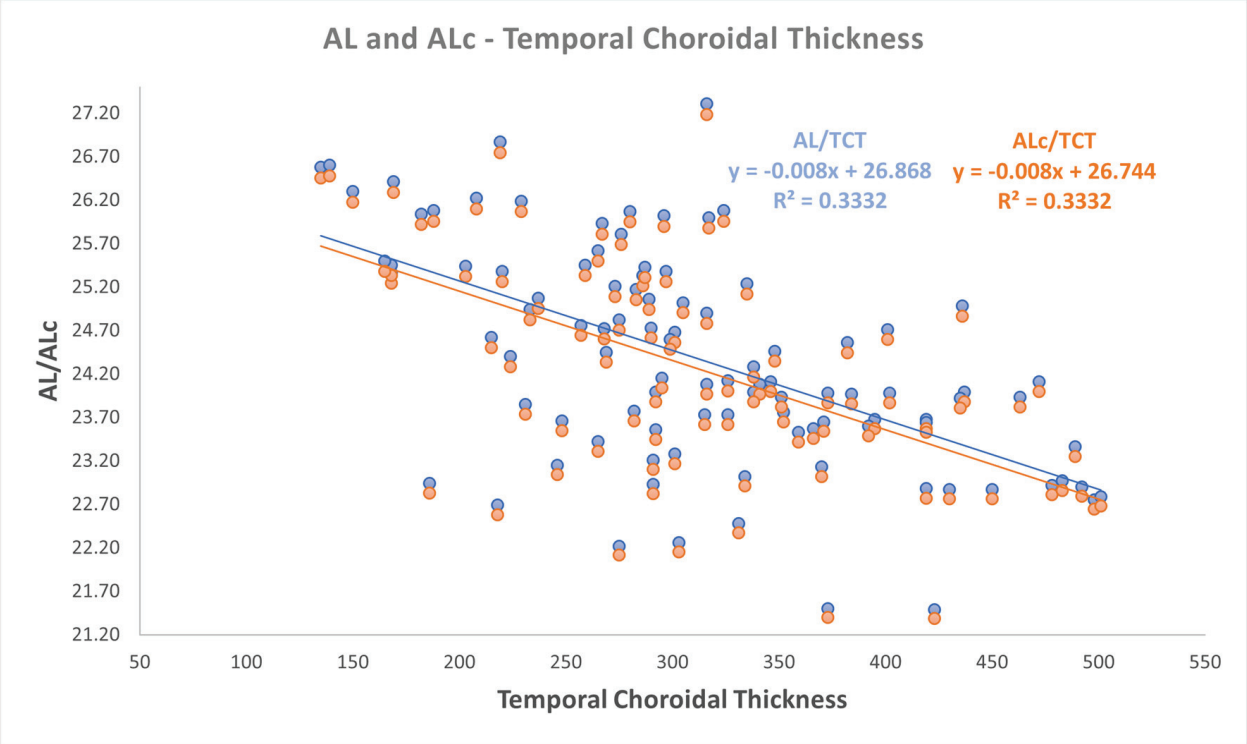

Figure 3. The relationship between both axial length (AL) and corrected AL (ALc) with temporal choroidal thickness.

Table 2. Correlation between uncorrected and corrected axial length and choroidal parameters.

Correlation	r	95% CI	R^2	*p*-Value
AL—Subfoveal ChT	−0.527	−0.669 −0.383	0.3372	0.000
AL—Nasal ChT	−0.581	−0.705 −0.449	0.2779	0.000
AL—Temporal ChT	−0.577	−0.700 −0.451	0.3332	0.000
ALc—Subfoveal ChT	−0.527	−0.669 −0.383	0.3372	0.000
ALc—Nasal ChT	−0.581	−0.705 −0.449	0.2779	0.000
ALc—Temporal ChT	−0.577	−0.700 −0.451	0.3332	0.000

AL: axial length, ALc: corrected axial length according to De Bernardo et al.; ChT: choroidal thickness; r: correlation coefficient according to Pearson correlation analysis; 95% CI: 95% confidence interval around r according to Pearson correlation analysis; R^2: coefficient of determination according to Pearson correlation analysis, *p*-value: level of significance according to Pearson correlation analysis.

Also, subgroups according to eye sides were normally distributed ($p > 0.050$) and the additional evaluation according to eye side subgroups is reported in Table 3.

Table 3. Correlation between uncorrected and corrected axial length and choroidal parameters in right eye and left eye subgroups.

Correlation	r—RE (*p*-Value)	95% CI—RE	r—LE (*p*-Value)	95% CI—LE
AL—Subfoveal ChT	−0.548 (0.000)	−0.727 −0.352	−0.621 (0.000)	−0.774 −0.412
AL—Nasal ChT	−0.546 (0.000)	−0.719 −0.334	−0.507 (0.000)	−0.697 −0.285
AL—Temporal ChT	−0.533 (0.000)	−0.711 −0.312	−0.624 (0.000)	−0.782 −0.421
Alc—Subfoveal ChT	−0.548 (0.000)	−0.727 −0.352	−0.621 (0.000)	−0.774 −0.412
ALc—Nasal ChT	−0.546 (0.000)	−0.719 −0.334	−0.507 (0.000)	−0.697 −0.285
Alc—Temporal ChT	−0.533 (0.000)	−0.712 −0.312	−0.624 (0.000)	−0.782 −0.421

RE: right eyes; LE: left eyes; AL: axial length, ALc: corrected axial length according to De Bernardo et al.; ChT: choroidal thickness; r: correlation coefficient according to Pearson correlation analysis; 95% CI: 95% confidence interval around r according to Pearson correlation analysis; *p*-value: level of significance according to Pearson correlation analysis.

4. Discussion

In the literature, a negative correlation between ChT and AL has been clearly described.

Bulut A et al. studied 53 myopic children's eyes, for SE up to −0.75 diopter, and compared them to 64 emmetropic ones, with SE between −0.50 and +0.50 D. Myopic eyes showed a significantly thinner choroid than the emmetropic control eyes in subfoveal site and in three additional sites carried out every 750 μm temporal (T1, T2 and T3) and nasal (N1, N2 and N3) to the fovea. Furthermore, in the same population including myopic and emmetropic children, ChT was negatively correlated with AL and positively correlated with SE [7].

Gupta P et al. focused on the distribution of choroidal thickness in 448 myopic eyes compared to 116 emmetropic ones and concluded that highly myopic eyes have a significantly thinner peripapillary choroid and showed a different distribution of thickness compared with the controls [8].

Sonoda S et al. examined a large (7500 μm width) choroidal area of 180 right eyes of 180 healthy volunteers with an AL range between 21.9 and 27.7 mm, showing no statistically significant correlation between AL and total choroidal area, luminal area and stromal area. However, the authors found an inverse correlation between AL and the ratio of the luminal to stromal area, thus considering the luminal area to be more affected by reduction than the stromal one [9].

El-Shazly A et al. elaborated on the effect of ocular parameters, such as spherical equivalent (SE) and axial length (AL), on ChT. They assessed the ChT of patients with different degrees of myopia. AL showed a significant positive correlation with SE (r = 0.90 and $p < 0.001$) but a negative correlation with subfoveal, 2 mm nasal, temporal, upper and lower ChTs (r = −0.78, −0.83, −0.75, −0.76, and −0.69, respectively) ($p < 0.001$). Moreover, they checked if the ChT is affected uniformly throughout the macula or at different rates in different macular regions. In fact, they observed the thinnest choroid in the 2 mm nasal and the thickest choroid in the 2 mm temporal in mild and moderate myopia, in the 2 mm lower in the sever myopia, and in the 2 mm upper in advanced myopia. SE and AL were discovered to be the most important determinants for subfoveal nasal and temporal

ChTs; the SE was the only determining factor for 2 mm upper ChT, while AL was the only determinant for 2 mm lower ChT [10].

Liu B et al. studied adult highly myopic eyes and confirmed AL as the most significant predictor of the sub foveal ChT [11].

All the above-mentioned studies analyzed studies according to AL groups or between specific AL cut-off values. However, these limits could be affected by the reliability of AL measurements. Firstly, authors evaluate AL correction related to IOL power calculation. In fact, to reduce the percentage of eyes with hyperopic refractive outcome, Wang et al. published several preoperative AL correction factors [19–21].

Cooke et al., with the so-called Cooke modified AL (CMAL), instead demonstrated that CMAL can simulate a sum-of-segments AL by using a biometer that works with GRI. In fact, it is well-known that GRI-based biometers give a shorter value of AL in short eyes and longer values of AL in long eyes [22].

Contrary to the Wang–Koch AL-correcting factors [19–22], both ALc and CMAL should be applied not only in long eyes, but in eyes of all AL ranges [18,22,29].

Therefore, ALc was proposed based on the influence of lens opacity and GRI on the reliability of the AL measurement: in this way, a correcting factor applied to preoperative AL could eliminate any systematic error biometry [18]. IOLMaster 500 measures AL by utilizing a GRI, meaning that it uses a mean refractive index to calculate AL [29], so it is not able to internally correct such a systematic error, because it is inherent to the instrument operating principle [18,28,29]. Similarly, ALc should be also applied for all phakic AL measurements for all other GRI-based biometers, independently from both AL acquisition technologies and specific AL ranges [29]. Hence, even if our study found the same relationship between AL with ChT and between ALc with ChT, the cut-off values should be changed with consideration of the reliability of AL. For example, it is known that "High myopia" is defined as the presence of myopic refractive error associated with AL $\geq$ 26.00 mm [30,31], but when performing studies that analyzed ChT in myopic eyes, the cut-off values should be arranged according to the corrected AL.

To sum up, several sources of error could affect the reliability of AL measurement:

- Inaccurate measurement technique: for example, contact ultrasound biometry is known to be unreliable;
- Principle of function of optical biometer: GRI-based biometers are more sensitive to AL measurement error, rather than sum-of-segments biometers;
- Eye length, with less reliable AL values with GRI-based biometers in case of long eyes.
- Lens opacity, that could affect reliability of all types of optical biometers, but especially GRI-based biometers.
- In all these cases, both clinicians and researchers should pay attention in both AL and ChT evaluation.

A bias in these types of studies can be represented by accommodation. In fact, in this scenario, changes in crystalline lens could contribute to AL overestimation [12]. Particularly, Woodman EC et al. observed both AL and ChT changes accompanying accommodation in myopes and emmetropes. At baseline, myopes had an average AL of 24.73 $\pm$ 1.04 mm (n = 33) and emmetropes of 23.37 $\pm$ 0.81 mm (n = 16), with a high significantly difference (p< 0.001) between them. They described choroidal thinning by 9 $\pm$ 18 μm in myopic subjects during accommodation and by 7 $\pm$ 22 μm in emmetropes ones, with a statistically significant difference between the two groups (p < 0.05). Changes in AL and ChT were shown to have a highly significant but weak negative association at analysis of covariance (p < 0.001, r^2 = 0.077, slope b = $-$0.321) [13].

Kaple D et al. likewise compared AL and ChT changes during accommodation in young adult emmetropes and myopes, especially at peripheral sites. Upon accommodation, the AL changes maximally on-axis (41 ± 17 μm) and minimally at the 20° nasal site (26 ± 17 μm). On the other hand, the overall ChT decreased by 21 ± 7 μm ($F1276 = 23.85$, $p < 0.001$). ChT decreased more in myopes (23 ± 11 μm) than emmetropes (17 ± 8 μm) ($p = 0.02$). The greatest decrease of 30 ± 14 μm occurred on-axis and the lowest one of 14 ± 14 μm occurred at 30° temporally. ChT changes account for about 60% of changes arising for AL: the authors concluded that AL and ChT changes during accommodation are not significantly correlated with any position [14].

This phenomenon could be explained by the action of the ciliary muscle, choroidal non-vascular smooth muscle, and choroidal blood flow. The ciliary muscle is involved in the accommodation process and its contraction could also be transferred to the choroid, causing choroidal drag and scleral circumference reduction. Choroidal smooth muscle and blood flow could change in tone during accommodation, contributing to this event [15–17].

This knowledge highlights the cause and the possible management of eventual refractive errors occurring after measuring AL with optical biometry. It suggests measuring AL immediately following accommodation or during accommodation with an instrument that also provides measurements of lens thickness.

As a vascular tunic of the ocular globe, choroid is richly vascularized and provides the oxygen and nutrients required for the retinal pigment epithelium and of the outer retina. For the same reason, it can be reached by toxins, autoantibodies, and immunocomplexes, and so it can be involved in the pathogenesis of various ocular disorders. Investigating the eventual adaptations of the negative correlation between ChT and AL in various pathologic cohorts can reveal new features of their pathogenesis. For example, De Bernardo M et al. confirmed the existence of a significant correlation between AL and all the choroidal parameters in a celiac group, except for CVI, in a study comparing the subfoveal ChT of celiac patients with healthy controls [2].

It was already proven that ALc played a clinically relevant role in IOL power calculation [18,29], and AL correction is also essential in different fields of biometry. In fact, several reasons can clarify the importance of utilizing ALc in ChT studies:

(1) Ignoring AL correction could introduce bias to the ChT evaluation of patients grouped by AL. It results in an unreliable estimation of ChT of patients with macular edema and subretinal fluid [23] and in a difficult comparison between these patients and their healthy controls. Grading and staging different ocular and systemic pathologies through ChT could be an interesting clinical and therapeutic skill, so its trustworthy use is decisive.
(2) AL correction is essential for both patients with cataracts and subjects that underwent GRI-based biometry, as discussed in previous paragraphs.
(3) Studies concerning myopia progression and ChT [32,33] should be carried out according to ALc. The choice of AL cut-off values should be taken according to corrected AL values.
(4) Not only AL cut-off values, but also evaluation of both AL and ChT changes, should be analyzed according to ALc [14,24,25].
(5) In addition, relationships between ChT and retinal diseases [34] should be analyzed with ALc values.
(6) In conclusion, patients' stratification according to AL should be performed with ALc in ChT studies [26].

It should be noted that ALc by De Bernardo et al. [18] was developed by noting an apparent reduction in AL in post cataract surgery eyes: on this basis, they verified that the

refractive index of the human lens could change according to the cataract grade, making AL measurement in GRI-based biometers unreliable [18]. De Bernardo et al. demonstrated the presence of a systematic error in AL measurement in all cataractous eyes, despite the AL range [18]. For this reason, lens thickness was not considered by ALc: this parameter could be unreliable in case of cataract. ALc has more strength in eyes with cataract, therefore it should be considered, especially in case of examination of cataractous eyes. The OCT examinations were performed without any mydriatic eye drops. This should be considered a point of strength of this study. In fact, a significant thinning of the ChT in the temporal sector following the administration of tropicamide + phenylephrine has been demonstrated [23]. Such thinning was not observed in the subfoveal or nasal zone, probably because the presence of the optic nerve in the nasal choroidal side could influence the ChT, or due to an asymmetric choroidal watershed and choroidal vessels in the temporal choroid. For these reasons, the nasal and subfoveal ChT are not affected by mydriatics [23]. Avoiding pharmacological mydriasis has guaranteed the maximum reliability of ChT data, even in the temporal sector.

Since AL measurement with GRI-based biometry is inaccurate because they assume a global refractive index for different structures (cornea, aqueous, lens, vitreous), ChT measurements should not be influenced by GRI, but it was demonstrated that brightness of OCT images can influence the measurements of choroidal parameters. Therefore, ChT should not be corrected but OCT evaluation must be performed by using the same parameters [35]. Furthermore, OCT's light source is also a specific wavelength of light, thus it is also affected by the choroidal refractive index; therefore caution should be taken, especially when comparing different OCT devices in evaluation of choroidal parameters.

This study is affected by some limitations. Firstly, we evaluated both eyes for each subject. In fact, ocular measurements are more alike between fellow eyes. This similarity could cause a compounding of data; therefore, analyzing fellow eyes cannot be treated as if they were independent [36]. To overcome this problem, appropriate statistical analyses could be performed, such as the bootstrap or generalized estimating equations [36]. If the correlation between the left and right eyes of each patient is not accounted for in the statistical analysis, errors may occur in the obtained results: in fact, ignoring inter-eye correlation can lead to smaller *p*-values when both eyes are in the same group [36]. Another solution could be represented by the choice of using only one eye for each subject [36]. For this reason, we performed an additional analysis with bootstrap by evaluating subgroups based on the eyes' side (Table 3) and similarly a strong negative linear correlation was found both with AL and ALc compared to ChT (all $r < -0.500$, all $p < 0.050$).

In addition, the relationship between CMAL and ChT was not evaluated because the lens thickness (LT) measurement, required for CMAL [22], was not available with IOL Master 500. On the other hand, Wang–Koch adjustments were not analyzed because they were designed specifically to reduce hyperopic refractive error in long eyes [19–21].

A future prospective is represented by confirming the same relationship is also present in the case of "atypical" eyes: for example, it is well-known that refractive surgery causes a keratometry flattening, but also an AL, anterior chamber depth, and central corneal thickness (CCT) decrease [37,38]. Therefore, when the impact of corneal changes on IOL power calculation [37,38] and of CCT reduction on intraocular pressure measurement [39] are well-known, the impact of AL decrease on ChT evaluation is less well-known.

In conclusion, a statistically significant linear correlation persists between ALc and ChT, as in to the uncorrected AL. Due to systematic errors in GRI-based optical biometry, we recommend recruiting and stratifying population according to ALc, in order to apply the correct cut-off values in ChT studies. Therefore, both AL and ChT changes should be evaluated according to ALc.

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Data Availability Statement: The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. de Bernardo, M.; Altieri, V.; Coppola, A.; Gioia, M.; Rosa, N. Choroidal evaluation in patients under alpha-lytic therapy. *Graefe's Arch. Clin. Exp. Ophthalmol.* **2020**, *258*, 2729–2736. [CrossRef] [PubMed]
2. de Bernardo, M.; Vitiello, L.; Battipaglia, M.; Mascolo, F.; Iovino, C.; Capasso, L.; Ciacci, C.; Rosa, N. Choroidal structural evaluation in celiac disease. *Sci. Rep.* **2020**, *11*, 16398. [CrossRef] [PubMed]
3. Flores-Moreno, I.; Lugo, F.; Duker, J.S.; Ruiz-Moreno, J.M. The relationship between axial length and choroidal thickness in eyes with high myopia. *Am. J. Ophthalmol.* **2013**, *155*, 314–319. [CrossRef]
4. Spaide, R.F.; Koizumi, H.; Pozzoni, M.C. Enhanced depth imaging spectral-domain optical coherence tomography. *Am. J. Ophthalmol.* **2008**, *146*, 496–500, Erratum in *Am. J. Ophthalmol.* **2009**, *148*, 325. [CrossRef] [PubMed]
5. Fujiwara, T.; Imamura, Y.; Margolis, R.; Slakter, J.S.; Spaide, R.F. Enhanced depth imaging optical coherence tomography of the choroid in highly myopic eyes. *Am. J. Ophthalmol.* **2009**, *148*, 445–450. [CrossRef] [PubMed]
6. Jonas, J.B.; Spaide, R.F.; Ostrin, L.A.; Logan, N.S.; Flitcroft, I.; Panda-Jonas, S. IMI-Nonpathological Human Ocular Tissue Changes With Axial Myopia. *Investig. Ophthalmol. Vis. Sci.* **2023**, *64*, 5. [CrossRef] [PubMed]
7. Bulut, A.; Oner, V.; Buyuktarakcı, Ş.; Kaim, M. Associations between choroidal thickness, axial length and spherical equivalent in a paediatric population. *Clin. Exp. Optom.* **2016**, *99*, 356–359. [CrossRef]
8. Gupta, P.; Cheung, C.Y.; Saw, S.M.; Bhargava, M.; Tan, C.S.; Tan, M.; Yang, A.; Tey, F.; Nah, G.; Zhao, P.; et al. Peripapillary choroidal thickness in young Asians with high myopia. *Investig. Ophthalmol. Vis. Sci.* **2015**, *56*, 1475–1481. [CrossRef]
9. Sonoda, S.; Sakamoto, T.; Yamashita, T.; Uchino, E.; Kawano, H.; Yoshihara, N.; Terasaki, H.; Shirasawa, M.; Tomita, M.; Ishibashi, T. Luminal and stromal areas of choroid determined by binarization method of optical coherence tomographic images. *Am. J. Ophthalmol.* **2015**, *159*, 1123–1131. [CrossRef]
10. El-Shazly, A.A.; Farweez, Y.A.; ElSebaay, M.E.; El-Zawahry, W.M.A. Correlation between choroidal thickness and degree of myopia assessed with enhanced depth imaging optical coherence tomography. *Eur. J. Ophthalmol.* **2017**, *27*, 577–584. [CrossRef] [PubMed]
11. Liu, B.; Wang, Y.; Li, T.; Lin, Y.; Ma, W.; Chen, X.; Lyu, C.; Li, Y.; Lu, L. Correlation of subfoveal choroidal thickness with axial length, refractive error, and age in adult highly myopic eyes. *BMC Ophthalmol.* **2018**, *18*, 127. [CrossRef]
12. Atchison, D.A.; Smith, G. Possible errors in determining axial length changes during accommodation with the IOL Master. *Optom. Cision Sci.* **2004**, *81*, 283–286. [CrossRef] [PubMed]
13. Woodman, E.C.; Read, S.A.; Collins, M.J. Axial length and choroidal thickness changes accompanying prolonged accommodation in myopes and emmetropes. *Vision. Res.* **2012**, *72*, 34–41. [CrossRef] [PubMed]
14. Kaphle, D.; Schmid, K.L.; Suheimat, M.; Read, S.A.; Atchison, D.A. Central and peripheral choroidal thickness and eye length changes during accommodation. *Ophthalmic Physiol. Opt.* **2023**, *43*, 311–318. [CrossRef] [PubMed]
15. Tamm, E.; Lütjen-Drecoll, E.; Jungkunz, W.; Rohen, J.W. Posterior attachment of ciliary muscle in young, accommodating old, presbyopic monkeys. *Investig. Ophthalmol. Vis. Sci.* **1991**, *32*, 1678–1692.
16. Woodman-Pieterse, E.C.; Read, S.A.; Collins, M.J.; Alonso-Caneiro, D. Regional changes in choroidal thickness associated with accommodation. *Investig. Ophthalmol. Vis. Sci.* **2015**, *56*, 6414–6422. [CrossRef] [PubMed]
17. Hoseini-Yazdi, H.; Read, S.A.; Alonso-Caneiro, D.; Collins, M.J. Retinal OFF-pathway overstimulation leads to greater accommodation-induced choroidal thinning. *Investig. Ophthalmol. Vis. Sci.* **2021**, *62*, 5. [CrossRef]

18. de Bernardo, M.; Cione, F.; Capasso, L.; Coppola, A.; Rosa, N. A formula to improve the reliability of optical axial length measurement in IOL power calculation. *Sci. Rep.* **2022**, *12*, 18845. [CrossRef]
19. Wang, L.; Shirayama, M.; Ma, X.J.; Kohnen, T.; Koch, D.D. Optimizing intraocular lens power calculations in eyes with axial lengths above 25.0 mm. *J. Cataract. Refract. Surg.* **2011**, *37*, 2018–2027. [CrossRef] [PubMed]
20. Wang, L.; Koch, D.D. Modified axial length adjustment formulas in long eyes. *J. Cataract. Refract. Surg.* **2018**, *44*, 1396–1397. [CrossRef] [PubMed]
21. Wang, L.; Holladay, J.T.; Koch, D.D. Wang-Koch axial length adjustment for the Holladay 2 formula in long eyes. *J. Cataract. Refract. Surg.* **2019**, *45*, 117. [CrossRef] [PubMed]
22. Cooke, D.L.; Cooke, T.L. Approximating sum-of-segments axial length from a traditional optical low-coherence reflectometry measurement. *J. Cataract. Refract. Surg.* **2019**, *45*, 351–354. [CrossRef] [PubMed]
23. Gioia, M.; de Bernardo, M.; Pagliarulo, S.; Cione, F.; Mottola, F.F.; Marca, A.L.; de Pascale, I.; Albano, G.; Rosa, N. Evaluation of Tropicamide-Phenylephrine Mydriatic Eye Drop Instillation on Choroidal Thickness. *J. Clin. Med.* **2023**, *12*, 6355. [CrossRef]
24. Hansen, M.H.; Kessel, L.; Li, X.Q.; Skovgaard, A.M.; Larsen, M.; Munch, I.C. Axial length change and its relationship with baseline choroidal thickness—A five-year longitudinal study in Danish adolescents: The CCC2000 eye study. *BMC Ophthalmol.* **2020**, *20*, 152. [CrossRef] [PubMed]
25. Ulaganathan, S.; Read, S.A.; Collins, M.J.; Vincent, S.J. Daily axial length and choroidal thickness variations in young adults: Associations with light exposure and longitudinal axial length and choroid changes. *Exp. Eye Res.* **2019**, *189*, 107850. [CrossRef] [PubMed]
26. Xuan, M.; Wang, D.; Xiao, O.; Guo, X.; Zhang, J.; Yin, Q.; Wang, W.; He, M.; Li, Z. Choroidal Vascularity and Axial Length Elongation in Highly Myopic Children: A 2-Year Longitudinal Investigation. *Investig. Ophthalmol. Vis. Sci.* **2024**, *65*, 7. [CrossRef]
27. Zeppieri, M.; Marsili, S.; Enaholo, E.S.; Shuaibu, A.O.; Uwagboe, N.; Salati, C.; Spadea, L.; Musa, M. Optical Coherence Tomography (OCT): A Brief Look at the Uses and Technological Evolution of Ophthalmology. *Medicina* **2023**, *59*, 2114. [CrossRef]
28. Pathak, M.; Sahu, V.; Kumar, A.; Kaur, K.; Gurnani, B. Current Concepts and Recent Updates of Optical Biometry—A Comprehensive Review. *Clin. Ophthalmol.* **2024**, *18*, 1191–1206. [CrossRef] [PubMed]
29. Cione, F.; de Bernardo, M.; Di Paola, I.; Caputo, A.; Graziano, M.; Rosa, N. IOL power calculation in long eyes: Selection of the best axial length adjustement factor using the most common formulas. *Heliyon* **2024**, *10*, e36609. [CrossRef] [PubMed]
30. Stopyra, W.; Langenbucher, A.; Grzybowski, A. Intraocular Lens Power Calculation Formulas—A Systematic Review. *Ophthalmol. Ther.* **2023**, *12*, 2881–2902. [CrossRef] [PubMed]
31. Voytsekhivskyy, O.; Tutchenko, L. Comparison of the prediction accuracy of 13 formulas in long eyes. *Graefe's Arch. Clin. Exp. Ophthalmol.* **2023**, *261*, 2575–2583. [CrossRef] [PubMed]
32. Li, Z.; Cui, D.; Hu, Y.; Ao, S.; Zeng, J.; Yang, X. Choroidal thickness and axial length changes in myopic children treated with orthokeratology. *Cont. Lens Anterior Eye.* **2017**, *40*, 417–423. [CrossRef] [PubMed]
33. Wang, Z.; Chen, J.; Kang, J.; Niu, T.; Guo, L.; Fan, L. Axial Length Control Is Associated with a Choroidal Thickness Increase in Myopic Adolescents After Orthokeratology. *Eye Contact Lens.* **2023**, *49*, 512–520. [CrossRef]
34. Sato, M.; Minami, S.; Nagai, N.; Suzuki, M.; Kurihara, T.; Shinojima, A.; Sonobe, H.; Akino, K.; Ban, N.; Watanabe, K.; et al. Association between axial length and choroidal thickness in early age-related macular degeneration. *PLoS ONE* **2020**, *15*, e0240357. [CrossRef]
35. Rosa, N.; Gioia, M.; Orlando, R.; De Luca, M.; D'Aniello, E.; Fioretto, I.; Sannino, C.; De Bernardo, M. Impact of Brightness on Choroidal Vascularity Index. *J. Clin. Med.* **2024**, *13*, 1020. [CrossRef]
36. Hoffer, K.J.; Aramberri, J.; Haigis, W.; Olsen, T.; Savini, G.; Shammas, H.J.; Bentow, S. Protocols for studies of intraocular lens formula accuracy. *Am. J. Ophthalmol.* **2015**, *160*, 403–405. [CrossRef] [PubMed]
37. Anders, P.; Anders, L.M.; Barbara, A.; Szentmary, N.; Langenbucher, A.; Gatzioufas, Z. Intraocular lens power calculation in eyes with previous corneal refractive surgery. *Ther. Adv. Ophthalmol.* **2022**, *14*, 25158414221118524. [CrossRef] [PubMed]
38. Anter, A.M.; Bleeker, A.R.; Shammas, H.J.; Suraneni, S.; Kingrey, B.; Murphy, D.A.; Leal, S.; Ghalibafan, S.; Tonk, R.S.; Cooke, D.L.; et al. Comparison of Legacy and New No-History IOL Power Calculation Formulas in Postmyopic Laser Vision Correction Eyes. *Am. J. Ophthalmol.* **2024**, *264*, 44–52. [CrossRef] [PubMed]
39. Albis-Donado, O.; Ramirez-Neria, P.; Rios-Acosta, N.; Stalmans, I. The influence of altitude on the differences between Goldmann tonometry and Pascal dynamic contour tonometry: An ecological meta-analysis. *Indian. J. Ophthalmol.* **2024**, *72* (Suppl. S3), S398–S403. [CrossRef] [PubMed]

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Evaluating the Feasibility of a Telescreening Program for Retinopathy of Prematurity (ROP) in Denmark

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Abstract: Objectives: This study investigates the feasibility of implementing telescreening for retinopathy of prematurity (ROP) using the ICON GO® widefield camera operated by a non-physician healthcare professional (NPHP). We hypothesized that images captured by an NPHP are adequate to evaluate ROP changes without further examinations. Secondly, the level of agreement between independent ROP graders were evaluated based on the fundus photographs. **Methods**: National ROP screening criteria were gestational age (GA) < 32 weeks or birthweight (BW) < 1500 g. Exclusion criteria were children hospitalized and born outside the Capital Region and examinations not performed by an NPHP. The screenings were performed using the ICON GO®. The NPHP selected the best images for evaluation by an *on*-site ophthalmologist, regarding whether re-examination was necessary and if so, whether the re-examination was beneficial. Lastly, the images were re-evaluated by an independent *off*-site ophthalmologist. **Results**: A total of 415 screening sessions on 165 patients performed by an NPHP were included. Re-examination was necessary in three screening sessions and beneficial in two. The level of agreement between the *on*-site and *off*-site ophthalmologists regarding ROP screening outcome was $k = 0.82$, ROP stage $k = 0.69$, plus disease $k = 0.69$, and lastly ROP zone $k = 0.37$. Of the screened children, ninety-seven (58.8%) had no ROP at any time points, sixty-two (37.6%) had some stage of ROP not requiring treatment, and six (3.6%) received ROP treatment. **Conclusions**: Telemedicine screening for ROP with the ICON GO® camera performed by an NPHP was feasible with an almost-perfect agreement and negligible need for re-examinations. The approach effectively identified children needing treatment, supporting the use of telescreening in ROP management.

Keywords: telescreening; prematurity; retinopathy of prematurity; imaging; non-physician healthcare professional

1. Introduction

Retinopathy of prematurity (ROP) is a vision-threatening retinal disease seen in children born preterm with low birth weight (BW) [1–3]. ROP is caused by a disturbance in the development of the immature eye [4]. In Denmark, screening for ROP has been performed for many years by highly specialized ophthalmologists using binocular indirect ophthalmoscopy (BIO) [5]. Another and more recent ROP screening method is using a camera, where wide-angle retinal photographs are captured. Screening with a camera enables telescreening consultation by recording and sending electronic image files of the patient's eyes [6]. This approach allows the patient's condition to be assessed remotely and at a later timepoint, while simultaneously enabling comparison of disease progression or regression over time. Classification of ROP is based on the current third edition established

by the International Classification of Retinopathy of Prematurity (ICROP3) [3]. The latest update of the classification was required in part due to advancements in modern technology. These advancements enable clinicians to observe more detailed retinal changes associated with ROP that need further attention.

Another advantage of telescreening for ROP is that it can be performed by non-physician healthcare professionals (NPHPs) and thus ensure a more efficient use of ophthalmological resources. In addition, screening with a camera may be less stressful for the children compared to BIO and the images can be stored and reviewed to ensure patient safety and enable quality studies and further research [7]. BIO is, however, still considered the gold standard of ROP screening [8,9]. A recently published study, which presented the latest advancements in ROP telemedicine, concluded that telemedicine can be an alternative to BIO [10]. However, it is important to note that there are huge differences in both the incidence and severity of ROP depending on the population studied. Furthermore, ROP telemedicine studies have employed a variety of designs (retrospective, prospective), methods (camera model, BIO as reference, digital BIO), professional performing the exam (ophthalmologist, non-physician), grader (ophthalmologist, non-physician, or AI), and outcome measures (severe ROP, presence of plus disease, ROP classification), where most have focused on the presence of plus disease [9,11–17].

Several original studies and a review have already compared screening outcomes between widefield cameras versus BIO [9,11,18,19]. This study focuses on comparing evaluations from independent ophthalmologists of images captured by an NPHP using exclusively a widefield camera.

The purpose of this study was to evaluate whether an NPHP can achieve reliable image quality in a new telemedicine setup for ROP in the Capital region of Denmark. We analyzed the frequency of re-examinations by an *on*-site ophthalmologist due to insufficient image quality. Additionally, we evaluated the level of agreement between independent ophthalmologists regarding ROP stage, zone, plus disease, and screening outcome.

2. Materials and Methods

2.1. Study Population and Recruitment

At the beginning of the study, the Danish national screening criteria for ROP was gestational age (GA) < 32 weeks or birthweight (BW) < 1500 g. Since April 2023, the national guidelines narrowed to GA < 32 weeks and BW < 1600 g. Screening starts no later than 5 weeks postnatally and at a post-menstrual age (PMA) of at least 31 weeks. The children were recruited as part of the clinical routine by healthcare staff involved in screening for ROP.

Children included in our study were born and hospitalized at one of the four neonatal intensive care units (NICUs) within the Capital region of Denmark. Children were included in the study in a one-year period from 5 September 2022 to 25 September 2023. The screening sessions included were performed by the NPHP using the ICON GO® camera (Phoenix ICON™ trademark of Neolight, LLC, Phoenix, AZ, USA). Exclusion criteria encompassed screening sessions performed using other methods than the ICON GO® or sessions conducted by an ophthalmologist on days when the NPHP was physically absent, such as during holidays or work leave. These exclusions were solely due to the absence of the NPHP, and not due to difficult cases or an inability to perform the screenings. The general condition of the child was always discussed with the team managing the child prior to the ROP screening to ensure that the child was fit for the examination. The ROP screening was postponed in cases where the neonatologists evaluated that the child was too weak, and if the *on*-site ophthalmologist could accept the level of risk by postponing the screening. The NPHP performed the screening sessions three days a week, with one of three *on*-site ophthalmologists available each day.

Fundus pigmentation was noted at the first screening session due to its potential impact on image processing and evaluation. Fundus pigmentation was categorized as defined by the American Academy of Ophthalmology as follows: light (choroidal vessels

visible within the macula), medium (choroidal vessels visible outside the arcades, but not within the macula), or dark (choroidal vessels not visible within the macula nor outside the arcades) [20].

2.2. Preparation of the Child Prior to ROP Examination

Local anesthesia was obtained using one drop of 0.4% oxybuprocaine, followed by two instillations with 10 min intervals of either 0.5% tropicamide and 2.5% phenylephrine or cyclomydrile (cyclopentolat hydrochlorid 0.2%/phenylephrin hydrochlorid 1%) applied by the neonatal nurse. After 30–45 min, the eyes were expected to be fully dilated. In cases of minimal effect, the eye drops were re-administered. Prior to inserting the eye speculum, local anesthesia was re-administered. Viscotears (Bausch & Lomb Nordic AB, Stockholm, Sweden) eye gel (2 mg/g carbomer) was administered for two main purposes: to lubricate and protect the cornea, and to serve as a bridge between the camera tip and the cornea.

To minimize the burden of the screening examinations, the children were swaddled and offered sugar water or breast milk, along with a pacifier, to help them stay calm and distracted. Vital signs of the child were monitored by the neonatal nurse assisting with the examination and screening was paused depending on the condition of the child.

2.3. Telescreening Setup

Before the study began, the NPHP completed five months of hands-on training under the supervision of an ROP expert (ophthalmologist). Additionally, the NPHP underwent retinal imaging training conducted by a clinical education specialist, who also reviewed images captured by the NPHP and who could approve the NPHP as a certified imaging technician. The clinical education specialist was from Neolight, Scottsdale, AZ, USA, which is a medical technology company providing solutions for neonatal care, for instance the photography device for ROP imaging as the ICON GO® (www.theneolight.com). The *on*- and *off*-site graders completed a case-based online training in grading ROP at the home page of the American Academy of Ophthalmology [21].

A portable wide-angled ICON GO® camera (Phoenix ICON™ trademark of Neolight, LLC, Phoenix, AZ, USA) was used for obtaining fundus photographs. Prior to each screening session, the ICON GO® was re-calibrated and the automated white balance function was set using a standard white background to ensure the level of brightness was equal to white during the recordings. The bedside setup consisted of an NPHP who was in the room with the child and a neonatal nurse. The dilating effect of the eyedrops was evaluated prior to the screening, and re-dilating drops were given if the pupil of either eye was < 6 mm in diameter. The NPHP recorded a video of the fundus of each eye. The initial video duration was set to a maximum of 90 s per eye. The examination was terminated either when the NPHP was satisfied with the images or when further technical improvement was not possible. From the video, the best frames per eye presenting all four quadrants and the central retina were chosen. The images were digitally exported to the Topcon IMAGEnet i-base V3.23.0 database (Topcon Europe Medical B.V., Capelle Aan De Ijssel, The Netherlands).

The *on*-site ophthalmologist was available at the same time and hospital where the screenings were performed, and reviewed the images outside the screening room. The *on*-site ophthalmologist decided if the image quality was sufficient to plan the screening outcome. The *on*-site ophthalmologist informed the parents on the screening results and outcome. In cases of insufficient images, the *on*-site ophthalmologist decided if the child should be re-examined and by which method (ICON GO® or BIO) to ensure patient safety. All images obtained during the screening session were re-evaluated by an independent *off*-site ophthalmologist within 3 days of the screening session. The *off*-site ophthalmologist worked from a separate office and did not have any direct contact with the patients. The *on*-site and *off*-site ophthalmologist were blinded to each other´s assessment. In cases of disagreement, the *off*-site ophthalmologist´s decision would overrule the *on*-site ophthalmologist. The *off*-site ophthalmologist was also the surgeon who would make the

final decision regarding Type 1 ROP treatment. There are no official guidelines regarding treatment for late ROP sequelae, hence these cases were discussed in case of disagreement. Information regarding the child's GA, BW, and PMA on the screening day was available for all ophthalmologists during the image evaluation, as were previously captured images in order to evaluate a possible regression or progression in ROP.

2.4. ROP Classification and Screening Outcome

ROP classification, including stage, zone, and plus disease, was assessed by the ophthalmologists for each eye of every patient according to the third International Classification of ROP [3]. All ophthalmologists used a standard guideline to determine the screening outcome based on the overall ROP evaluation of both eyes, as shown in Table 1. If one eye showed more severe clinical findings, the screening outcome was based on that eye. The ROP treatment criteria for Type 1 were based on the randomized clinical trial titled the Early Treatment for Retinopathy of Prematurity Study (ETROP) [22].

Table 1. Screening outcome depending on ROP classification.

Screening	Treatment
One-week-or-less Follow-up	Type 1 ROP
Zone I: immature vascularization, no ROP	
Zone I: Stage 1 or Stage 2 ROP	Zone I with plus disease
Immature retina extending into posterior Zone II	Zone I Stage 3 without plus
Suspected presence of AP-ROP	Zone II Stage 2 or 3 with plus disease
Stage 3 ROP	
One- to two-week Follow-up	A-ROP
Posterior Zone II: immature vascularization	A-ROP is characterized by the rapid onset of abnormal
Zone II: Stage 2 ROP	neovascularization and severe plus disease, occurring
Zone I: unequivocally regressing ROP	without the chronologic stage progression of ROP
Two-week Follow-up	Hybrid-ROP
Zone II: Stage 1 ROP	
Zone II: no ROP, immature vascularization	Stage 3 including A-ROP characteristics in same eye
Zone II: unequivocally regressing ROP	with plus disease
Two- to three-week Follow-up	
Zone III: Stage 1 or 2 ROP	
Zone III: regressing ROP	
Stop Screening	
PMA > 36 weeks AND no sign of ROP in earlier screenings in Zone I or entire Zone II	
Fully regressed ROP	

Note: Screening and treatment schedule inspired by Fierson et al. (2018) [23]. ROP classification following the current guidelines as defined by ICROP3 [3]. The definition of Hybrid-ROP is as described by Sanghi et al., 2012 [24].

2.5. Data Access and Management

The children's medical files were reviewed prior to each screening session to track where the child was hospitalized and to achieve a status of the general health condition of the child. Information on GA, BW, and PMA was extracted from the medical file. PMA and GA were converted from total days to weeks.

2.6. Statistical Methods

Descriptive statistics for GA, BW, and PMA were tested for normality. Data that were non-parametric or had a small sample size were analyzed using the Mann–Whitney test to assess differences between included and excluded patients. Normally distributed variables were analyzed using the unpaired *t*-test.

The screening outcome was defined as the final conclusion for each session, which included an overall evaluation of both eyes. However, the ROP classification was assessed separately for each eye of every patient by both on-site and off-site ophthalmologists. To simplify the results section, we tested for statistical differences between the eyes. If no significant differences were found between the right and left eyes, we would present the ROP classification for one eye only. Consequently, the kappa statistics were provided for the screening outcome (based on both eyes) and the ROP classification (based on one eye).

The level of agreement regarding the screening outcome and ROP classification (zone, stage, and presence of plus disease) was analyzed by Cohen's kappa. The weighted kappa was presented for each of the three *on*-site ophthalmologists compared with the *off*-site ophthalmologist, as well as for all *on*-site ophthalmologists collectively compared with the *off*-site ophthalmologist. The kappa statistic has a range of k < 0.00 to k = 1, sorted in six categories depending on the value. The strength of the agreement according to the kappa value is graded as poor (k < 0.00), slight (k = 0.00–0.20), fair (k = 0.21–0.40), moderate (k = 0.41–0.60), substantial k = 0.61–0.80), and almost perfect (k = 0.81–1.00) [25]. The *p*-values computed as part of the kappa statistics were not presented, because the assumption of no association was unreasonable [26] (pp. 433–437).

The Chi-square test was performed to analyze the statistical differences between the right and left eyes in the ROP classification (stage, zone, and plus disease) for each of the *on*-site and *off*-site graders. A *p*-value less than 0.05 was considered statistically significant. Data were collected in REDcap (Research Electronic Data Capture, Vanderbilt University, Nashville, TN, USA) and analyzed using Stata 18.5 (StataCorp LLC, College Station, TX, USA).

2.7. Approvals

The Danish Data Protection Agency approved the study (P-2020–1206). Ethical board approval was waived by The Danish National Medical Research Ethics Committee (VMK, decision number VMK-protokol_200921_V1). The legal department at Rigshospitalet decided that the study could be performed by approval from the local head of department.

3. Results

3.1. Study Population Characteristics

A total of 556 screening sessions were performed on 174 patients during the study period. The 141 (25%) screening sessions exclusively performed by an ophthalmologist were excluded from the study. Thus, the study was based on 415 screening sessions (75% of all performed screening sessions) performed by the NPHP on 165 children (53% males). Additionally, nine (5%) patients were exclusively examined by the on-site ophthalmologists, see Figure 1. We examined the data for inclusion bias and found that the excluded patients compared to the included had a significantly higher mean GA (30.9 $\pm$ 0.7 and 28.9 $\pm$ 2.4 weeks, p = 0.017), mean BW (1442.6 $\pm$ 176.7 and 1206.9 $\pm$ 394.3 g, p = 0.04), and mean PMA at the time of screening (38.01 $\pm$ 4.4 and 36.2 $\pm$ 2.6 weeks, p = 0.0003), respectively.

Each child was on average screened 2.5 times by the NPHP during the screening period, ranging from 1 to 12 screening sessions per patient. A total of 97 (58.8%) patients did not develop any ROP (Stage 0), whereas n = 68 (41.2%) were graded as having ROP (Stage 1–4A) during the screening period. ROP stage, using the most severe stage encountered for each patient during the screening period, was Stage 1: n = 3 (1.8%), Stage 2: n = 42 (25.5%), Stage 3: n = 21 (12.7%), A-ROP: n = 1 (0.6%), Stage 4A: n = 1 (0.6%), see Figure 2. The patient evaluated as having ROP Stage 4A was examined using ultrasound in full anesthesia

initially before treatment. Here, the surgeon found Stage 3 ROP in all four quadrants but retinal detachment was not observed. No patients were graded as having Stage 4B or 5.

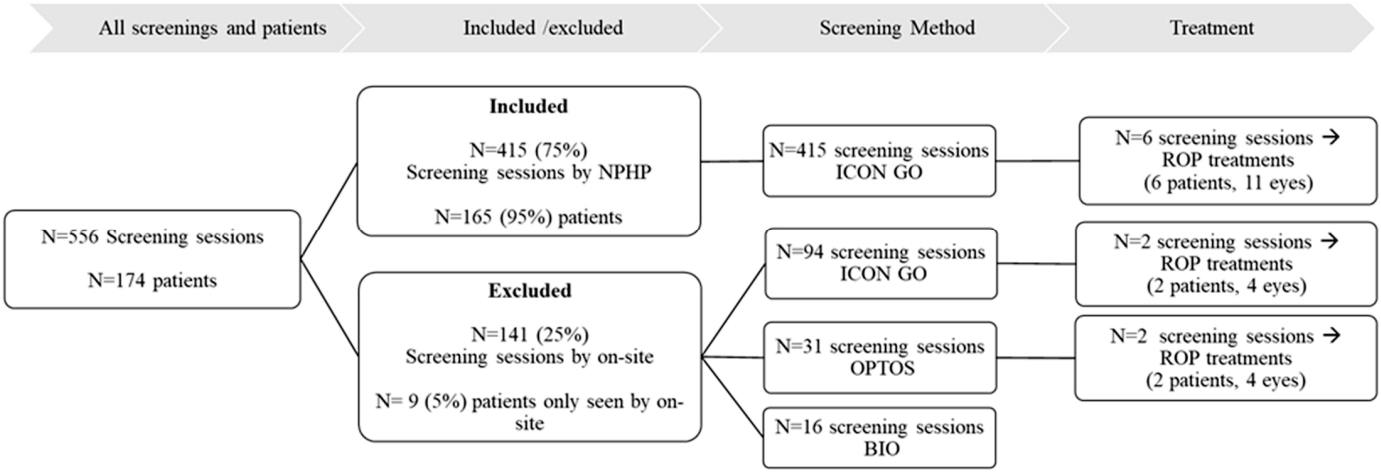

Figure 1. Flowchart of the included patients and screenings during study period. Nine patients received treatment on both eyes, and one patient was treated on the left eye only during the study period. Abbreviations: non-physician healthcare professional (NPHP), binocular indirect ophthalmoscopy (BIO), and retinopathy of prematurity (ROP).

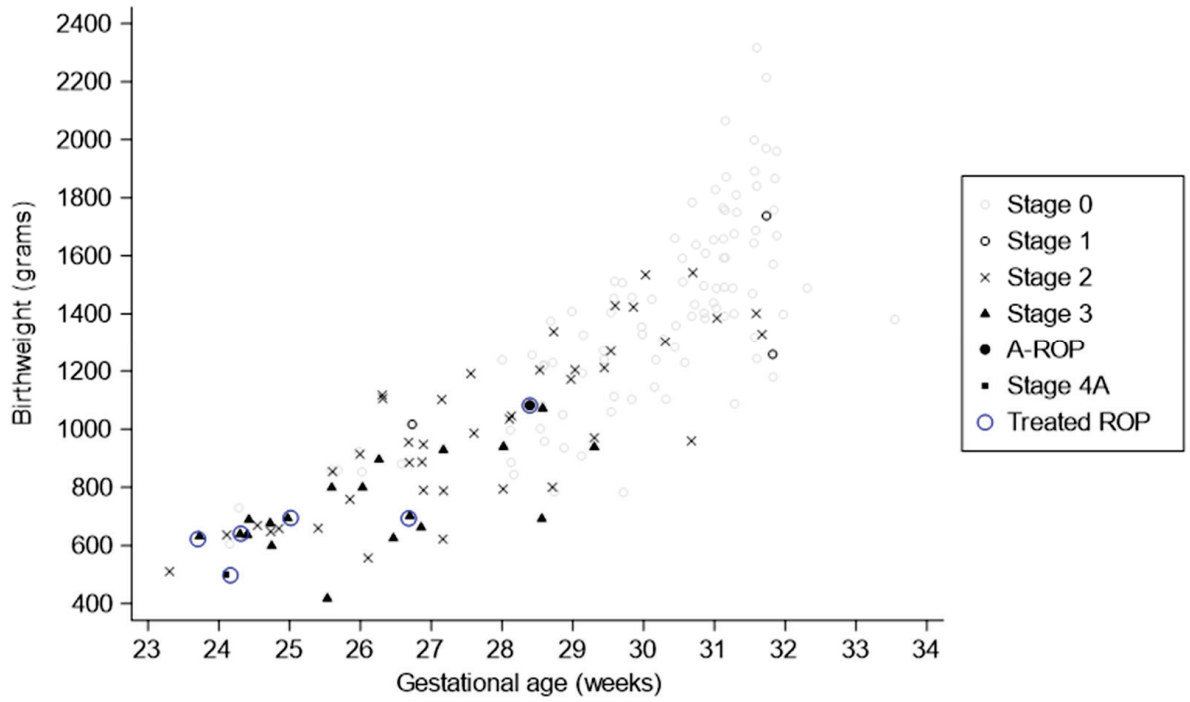

Figure 2. Scatterplot (jitter) of birthweight and gestational age for each patient differentiating the maximum stage of retinopathy of prematurity (ROP) detected during the screening period. Patients receiving treatment for ROP are marked with a blue open circle; the symbol inside the open circle is for the same patient.

Six patients (3.6%) (eleven eyes) included in the study received laser therapy for ROP, see Figure 2. The median PMA at the time of treatment was 36.3 weeks (IQR: 34.9–42.7, range: 33.6–46.7), see Table 2. The decision to treat was based on screening sessions performed by the NPHP, see Figure 1. Four out of six cases were due to Type 1 ROP and A-ROP. The remaining two cases received treatment due to structural changes that did not

meet the treatment criteria for Type 1 ROP (e.g., suspicion of retinal traction or fold with or without the presence of hemorrhages or persistent Stage 3 ROP), see Table 3.

Table 2. PMA (weeks) at maximum ROP stage during the study period.

	N (%)	PMA, Median	PMA, (Min–Max)
Any ROP	68 (41.2)	34.6	(31.3–46.7)
Stage 1	3 (1.8)	36.7	(32.1–36.7)
Stage 2	42 (25.5)	34.1	(31.3–39.4)
Stage 3	20 (12.7)	34.9	(31.9–46.7)
A-ROP	1 (0.6)	34.9	(34.9–34.9)
Stage 4A	1 (0.6)	42.7	(42.7–42.7)
Treatment for ROP	6 (3.6)	36.3	(33.6–46.7)

Note: Post-menstrual age (PMA) in weeks tabulated with the maximum stage of retinopathy of prematurity (ROP) during the screening period. No children had re-activated ROP or Stage 4B or 5. PMA at the first treatment for ROP is also presented. Patients who did not develop ROP during the study (n = 97, 58.8%) are not presented in this table.

One patient received treatment on one eye only. Five patients were treated on both eyes; in one of those eyes, the last treatment was performed outside the study period at a PMA of 41 + 4. Additionally, one patient had a maximum Stage 0 seen in Zone II without plus during the study period but received treatment after the end of the study due to progression to post Zone II Stage 3 with plus disease. The screening sessions during the study period but prior to the development of treatment warranting ROP are included in the kappa analyses.

Table 3. Specifications of patients who received treatment for ROP.

ID	GA	BW	ROP Classification and Outcome	Indication	PMA	Weight
166	23 + 5	628	***On*-site** LE: Post. Zone II. Stage 3. Plus Plan: Treatment LE ***Off*-site** LE: Post. Zone II. Stage 3. Plus Plan: Treatment LE	Type 1	36 + 4	2505
172	26 + 5	698	***On*-site** RE: Zone II. Stage 3. Pre-plus LE: Zone II. Stage 3. Pre-plus Plan: Treatment BE ***Off*-site** RE: Post. Zone II. Stage 3. Pre-plus LE: Post. Zone II. Stage 3. Plus Plan: Treatment BE	Type 1	36 + 2	2030
173	25 + 0	698	***On*-site** RE: Zone II. Stage 3. Plus LE: Zone II. Stage 3. Plus Plan: Treatment BE ***Off*-site** RE: Post. Zone II. Stage 3. Plus LE: Post. Zone II. Stage 3. Plus Plan: Treatment BE	Type 1	33 + 5	2080
12	28 + 3	1082	***On*-site** RE: Zone I by notch A-ROP. No plus Plan: Treatment RE ***Off*-site** RE: Zone I by notch A-ROP. No plus Plan: Treatment RE	Other Rapid ROP progression with hemorrhages	35 + 0	1915

Table 3. *Cont.*

ID	GA	BW	ROP Classification and Outcome	Indication	PMA	Weight
66	24 + 1	500	***On*-site** RE: Zone II. Stage 3. Pre-plus LE: Zone II. Stage 4A. Pre-plus Plan: Treatment BE ***Off*-site** RE: Zone II. Stage 3. Pre-plus LE: Zone II. Stage 3. Pre-plus Plan: Screening $\leq$ 1 week	Other worrisome structural ROP changes	42 + 6	3256
127	24 + 2	640	***On*-site** RE: Zone II. Stage 3. Pre-plus LE: Zone II. Stage 3. Pre-plus Plan: Screening $\leq$ 1 week ***Off*-site** RE: Post Zone II. Stage 3. Hybrid ROP Pre-plus LE: Zone II. Stage 3. Pre-plus Plan: Treatment BE	Other worrisome structural ROP changes	47 + 5	4100

Note: This table summarizes the patients, screened by the non-physician healthcare professional (NPHP), who received laser therapy treatment for ROP as the first treatment. Abbreviations: right eye (RE), left eye (LE), both eyes (BE), gestational age (GA), birth weight (BW), retinopathy of prematurity (ROP), and post-menstrual age (PMA).

The majority n = 107 (65%) had a lightly pigmented fundus, followed by 42 (25%) with a medium pigmented fundus, and lastly 16 (10%) had a dark fundus. Forty-five children (27%) were twins (twenty-two twin pairs, where one twin was excluded because screening was performed only by the on-site ophthalmologist).

3.2. Correlation between Image Quality, Satisfaction, and Fundus Pigmentation

In three (0.7%) screening sessions, the on-site ophthalmologist deemed the images unsatisfactory, while the NPHP had only flagged two of these sessions as unsatisfactory. In the first case, BIO was performed by the on-site ophthalmologist enabling visualization of the ridge, while the NPHP was similarly unsatisfied due to technical difficulties in imaging a darkly pigmented retina. In the second case, the on-site ophthalmologist was unsure whether there was a hemorrhage or an artifact on the images and performed BIO to rule out hemorrhages, while the NPHP was likewise not satisfied with the visualization of Zone II. In the last case, the on-site ophthalmologist supplemented the images with BIO but did not obtain new information; comparatively, the NPHP was satisfied with the image quality.

The off-site ophthalmologist was unsatisfied with two (0.5%) screening sessions. In one case, not all the images had been correctly uploaded to the imaging program; in another case, the ridge was not sufficiently visualized. The screening sessions deemed unsatisfactory by the on-site and off-site ophthalmologists did not overlap. The likelihood of deeming images unsatisfactory increased with greater retinal pigmentation for both the NPHP (p = 0.0001) and the on-site ophthalmologist (p = 0.014). However, this trend was not observed for the off-site ophthalmologist (p = 0.26).

3.3. Agreement on Screening Outcome between On-Site and Off-Site Ophthalmologists

The outcome of the screening session could be to treat ROP, screen again in $\leq$1 week, 1–2 weeks, 2 weeks, 2–3 weeks, or terminate the screening, based on the worst eye of each patient. We evaluated the level of agreement for the screening outcome between three on-site and one off-site ophthalmologists and found an 'almost-perfect' agreement between all ophthalmologists (k = 0.82, weighted kappa). The screening outcome deviated by one level in sixty-seven (16%) screening sessions, two levels in twenty (5%), and three levels in two (0.5%) screening sessions, see Table 4. No screening outcomes deviated by four or more levels. Although the frequency of disagreement regarding screening outcomes was slightly higher in darkly pigmented retinas, it was not significant. Overall, the frequency of

agreement and disagreement did not vary significantly across the different pigmentation levels of light (79.2% and 20.8%), medium (79.3% and 20.7%), or dark (74% and 26%), respectively ($p = 0.69$).

Table 4. Level of agreement for screening outcome based on the most affected eye if unequal.

		***Off*-Site**						
		Treat	**≤1 w**	**1–2 w**	**2 w**	**2–3 w**	**Terminate**	**Total**
***On*-site 1**	**Treat**	4	1	0	0	0	0	5
	≤1 w	0	33	5	0	0	0	38
	1–2 w	0	3	37	4	0	1	45
	2 w	0	0	11	33	0	4	48
	2–3 w	0	0	1	1	0	0	2
	Terminate	0	0	1	2	0	25	28
	Total	4	37	55	40	0	30	166
***On*-site 2**	**Treat**	0	0	0	0	0	0	0
	≤1 w	0	4	8	0	0	0	12
	1–2 w	0	0	27	5	0	0	32
	2 w	0	0	6	41	0	4	51
	2–3 w	0	0	1	0	0	3	4
	Terminate	0	0	0	2	0	49	51
	Total	0	4	42	48	0	56	150
***On*-site 3**	**Treat**	0	0	0	0	0	0	0
	≤1 w	1	12	5	0	0	0	18
	1–2 w	0	0	19	1	0	0	20
	2 w	0	0	5	16	0	1	22
	2–3 w	0	0	2	6	2	2	12
	Terminate	0	0	0	3	0	24	27
	Total	1	12	31	26	2	27	99
***On*-site all**	**Treat**	4	1	0	0	0	0	5
	≤1 w	1	49	18	0	0	0	68
	1–2 w	0	3	83	10	0	1	97
	2 w	0	0	22	90	0	9	121
	2–3 w	0	0	4	7	2	5	18
	Terminate	0	0	1	7	0	98	106
	Total	5	53	128	114	2	113	415

	Agreement	Expected agreement	Kappa, wgt (95% CI)	SE
On-site 1 × *off*-site	94.58%	69.79%	0.82 (0.73–0.88)	0.05
On-site 2 × *off*-site	94.00%	63.80%	0.83 (0.79–0.86)	0.06
On-site 3 × *off*-site	93.54%	67.69%	0.80 (0.77–0.84)	0.07
ALL × *off*-site	94.55%	68.40%	**0.82 (0.82–0.85)**	0.04

Note: Screening outcome tabulated between the *on*-site and *off*-site ophthalmologists' evaluation based on either eye with the most severe retinopathy of prematurity (ROP). The highlighted gray cells are decisions with full agreement. Abbreviations: week (w), weighted (wgt), confidence interval (CI), and standard error (SE).

3.4. Agreement on ROP Classification

Stage, zone, and plus disease did not differ significantly between the right and left eyes for each of the on- and off-site graders ($p > 0.05$). Data in this section are therefore presented for the right eye only.

Stage was categorized as Stage 0, Stage 1, Stage 2, Stage 3, A-ROP, and regression ROP. The level of agreement for ROP stage between the off-site and three on-site ophthalmologists was substantial (weighted kappa k = 0.69). ROP stage deviated by one level in twenty-eight (6%) screening sessions, two levels in twenty-three (5%), three levels in twenty-one (5%), four levels in two (0.5%), and by five levels in the last fifteen (3%) screening sessions, see Table 5.

Table 5. Level of agreement of ROP stage—Right eye.

		***Off*-Site**						
		Stage 0	**Stage 1**	**Stage 2**	**Stage 3**	**A-ROP**	**Reg ROP**	**Total**
***On*-site 1**	**Stage 0**	69	1	3	1	0	1	75
	Stage 1	0	1	0	0	0	0	1
	Stage 2	5	2	32	5	0	0	44
	Stage 3	1	0	5	21	0	2	29
	A-ROP	0	0	0	0	1	0	1
	Reg ROP	0	1	5	0	0	9	15
	Total	75	5	45	27	1	12	165
***On*-site 2**	**Stage 0**	100	0	2	0	0	3	105
	Stage 1	2	1	0	0	0	0	3
	Stage 2	8	1	19	0	0	1	29
	Stage 3	0	0	3	0	0	0	3
	A-ROP	0	0	0	0	0	0	0
	Reg ROP	3	0	5	0	0	2	10
	Total	113	2	29	0	0	6	150
***On*-site 3**	**Stage 0**	43	0	0	0	0	0	43
	Stage 1	0	2	0	0	0	0	2
	Stage 2	1	0	20	2	0	1	24
	Stage 3	0	0	4	9	0	0	13
	A-ROP	0	0	0	0	0	0	0
	Reg ROP	7	1	5	0	0	4	17
	Total	51	3	29	11	0	5	99
***On*-site all**	**Stage 0**	212	1	5	1	0	4	223
	Stage 1	2	4	0	0	0	0	6
	Stage 2	14	3	71	7	0	2	97
	Stage 3	1	0	12	30	0	2	45
	A-ROP	0	0	0	0	1	0	1
	Reg ROP	10	2	15	0	0	15	42
	Total	239	10	103	38	1	23	414

	Agreement	Expected agreement	Kappa, wgt (95% CI)	SE
On-site 1 × *off*-site	92.36%	66.23%	0.77 (0.77–0.83)	0.06
On-site 2 × *off*-site	89.67%	74.94%	0.59 (0.52–0.61)	0.07
On-site 3 × *off*-site	87.12%	60.56%	0.67 (0.65–0.74)	0.07
ALL × *off*-site	90.24%	68.45%	**0.69 (0.64–0.79)**	0.04

Note: Retinopathy of prematurity (ROP) stage tabulated between the on-site and off-site ophthalmologists for the right eyes. Stage evaluations with full agreement are marked in grey. Abbreviations: aggressive ROP (A-ROP), regressed ROP (reg ROP), weighted (wgt), confidence interval (CI), and standard error (SE).

Zone was categorized as Zone I by notch, Zone I, posterior Zone II, Zone II, and Zone III. The weighted kappa between the one off-site and three on-site ophthalmologists

was k = 0.37, equivalent to a fair agreement. The frequency of deviations by one level was *n* = 92 (22%) and by two levels was *n* = 1 (0.2%), see Table 6.

Table 6. Level of agreement of ROP Zone—Right eye.

		***Off*-Site**					
		Zone I Notch	**Zone I**	**Post Zone II**	**Zone II**	**Zone III**	**Total**
***On*-site 1**	**Zone I notch**	1	0	0	0	0	1
	Zone I	1	2	2	0	0	5
	Post Zone II	0	1	10	3	0	14
	Zone II	0	1	41	101	0	143
	Zone III	0	0	0	2	0	2
	Total	2	4	53	106	0	165
***On*-site 2**	**Zone I notch**	1	0	0	0	0	1
	Zone I	0	0	2	0	0	2
	Post Zone II	0	0	6	9	0	15
	Zone II	0	0	3	126	0	129
	Zone III	0	0	0	3	0	3
	Total	1	0	11	138	0	150
***On*-site 3**	**Zone I notch**	0	0	0	0	0	0
	Zone I	0	0	2	0	0	2
	Post Zone II	0	0	1	5	0	6
	Zone II	0	0	5	73	0	78
	Zone III	0	0	0	13	0	13
	Total	0	0	8	91	0	99
***On*-site all**	**Zone I notch**	2	0	0	0	0	2
	Zone I	1	2	6	0	0	9
	Post Zone II	0	1	17	17	0	35
	Zone II	0	1	49	300	0	350
	Zone III	0	0	0	18	0	18
	Total	3	4	72	335	0	414

	Agreement	Expected agreement	Kappa, wgt (95% CI)	SE
On-site 1 × *off*-site	92.12%	87.69%	0.36 (0.26–0.46)	0.05
On-site 2 × *off*-site	97.17%	93.99%	0.53 (0.41–0.59)	0.06
On-site 3 × *off*-site	91.58%	90.00%	0.16 (0.10–0.36)	0.05
ALL × *off*-site	94.32%	91.06%	**0.37 (0.37–0.40)**	0.04

Note: Retinopathy of prematurity (ROP) zone tabulated between the *on*-site and *off*-site ophthalmologists for the right eyes. Abbreviations: weighted (wgt), confidence interval (CI), and standard error (SE).

Plus disease was categorized as no plus, pre-plus, and plus. The weighted kappa regarding the presence of plus disease for the right eye was k = 0.69, corresponding to a substantial level of agreement. Deviations by one level were *n* = 24 (6%) on the right eyes, and *n* = 25 (6%) on the left eyes, when comparing all three ophthalmologists, see Table 7. No deviations by two levels occurred (plus versus no plus).

Table 7. Level of agreement of the presence of plus disease—Right eye.

		Off-Site			
		No Plus	**Pre-Plus**	**Plus**	**Total**
***On*-site 1**	**No plus**	134	11	0	145
	Pre-plus	3	16	0	19
	Plus	0	0	1	1
	Total	137	27	1	165
***On*-site 2**	**No plus**	144	3	0	147
	Pre-plus	1	2	0	3
	Plus	0	0	0	0
	Total	145	5	0	150
***On*-site 3**	**No plus**	82	2	0	84
	Pre-plus	4	11	0	15
	Plus	0	0	0	0
	Total	86	13	0	99
***On*-site all**	**No plus**	360	16	0	376
	Pre-plus	8	29	0	37
	Plus	0	0	1	1
	Total	368	45	1	414
	Agreement	Expected agreement	Kappa, wgt (95% CI)		SE
On-site 1 × *off*-site	95.76%	86.91%	0.68 (0.61–0.73)		0.07
On-site 2 × *off*-site	97.33%	94.80%	0.49 (0.06–0.92)		0.08
On-site 3 × *off*-site	93.94%	75.70%	0.75 (0.56–0.94)		0.10
ALL × *off*-site	94.20%	81.70%	**0.69 (0.59–0.77)**		0.05

Note: Presence of plus disease evaluation tabulated between the *on*-site and *off*-site ophthalmologists for the right eyes. Abbreviations: weighted (wgt), confidence interval (CI), and standard error (SE).

4. Discussion

4.1. Main Findings

In this paper, we investigated the feasibility of an NPHP performing ROP screening on prematurely born babies using wide-angle fundus photographs obtained by the ICON GO® camera. Images were evaluated independently by three *on*-site and one *off*-site ophthalmologist. The *on*-site ophthalmologist was present outside the screening room to ensure the safety of the study in cases where screening by the NPHP was deemed insufficient to reach a decision on the screening session outcome. Re-screening by the *on*-site ophthalmologist was performed in three out of four hundred and fifteen screening sessions and in two out of three, the re-examination was beneficial for reaching a decision. Photographs were re-evaluated by an *off*-site ophthalmologist who also had the final decision for the treatment of Type 1 ROP. There was an 'almost-perfect' level of agreement between the *on*-site and *off*-site ophthalmologists regarding the screening outcome, assessed by Cohen's kappa, followed by ROP stage and presence of plus disease, and lastly by ROP Zone in a descending order.

4.2. Defining Good Image Quality

High-quality images are important in order to classify ROP and decide the screening outcome. In our study, the certified NPHP was focused on image quality, including brightness, focus, and visualization of the retinal periphery. The degree of pigmentation of the ocular background played a significant role in the ease of obtaining images of sufficient quality as observed elsewhere [20]. The ophthalmologists had the possibility to improve the image brightness and contrast in the viewing software which was unavailable to the NPHP during the screening session. This indicates that slightly lower quality images may be still

useful to make an adequate assessment, which supports the low rate of re-examinations observed in our study [12]. It is crucial to establish a standardized training program for NPHPs who operate widefield cameras during these screenings. The e-ROP study developed a thorough training program that included guidelines for imaging, selecting, and exporting retinal images [27]. This program can be adapted as a model for ROP telescreening, helping to ensure consistent and accurate results.

4.3. Potential Inclusion Bias

Our analysis indicated that the excluded patients were older than the included patients. These findings support the unequal distribution of ROP zone between the groups, with a higher frequency of Zone III involvement in the excluded screening sessions. These results were enhanced by the superior ability of examining Zone III with BIO compared to the camera. In summary, patients at high risk of acute ROP were primarily examined by the NPHP and the study results might overrepresent the most severe cases. Thus, considering the aim of the project, evaluating the feasibility of ROP telescreening performed with the ICON GO®, we do not believe that potential inclusion bias has a negative impact on the clinical implications of our findings.

4.4. Discrepancies in ROP Classification between Ophthalmologists

This is the first Danish study to analyze the feasibility and setup of a telescreening program in detecting ROP performed by an NPHP. Studies have been conducted elsewhere with non-physicians as the technicians [11–13,15,28,29]. These studies have shown promising results with some degree of variability in ROP classification, but a good level of agreement regarding ROP requiring treatment. The study by Campbell et al. presented the total percentage of image-based discrepancies between two experts for zone, stage, and plus disease, 8%, 40%, and 18%, respectively [15]. In comparison, our study showed discrepancies of 22.5% for zone, 19.5% for stage, and 5.7% for plus disease. The inconsistency between the studies can be explained by the fact that more categories were included in our study. For instance, the zone was categorized in I, II, and III, whereas we additionally had Zone I by notch and posterior Zone II. The agreement on defining the ROP zone was notably inconsistent in our study. Zone I is defined as a circular area centered on the optic nerve, with a radius equal to twice the distance from the macula to the optic disc center [3]. This definition does not account for the ocular growth during the neonatal period. A study aimed at predicting the Zone I area found that changes in axial length had the most significant impact [30]. This finding may help explain the discrepancies in ROP zone evaluations. Adopting a standardized template for defining Zone I based on the PMA of the child could potentially improve consistency in future research.

A previous Danish study investigated the inter-rater agreement regarding particularly plus disease. The available fundus photographs captured prior to ROP treatment were evaluated retrospectively by four experts. The level of agreement was inadequate for plus disease; however, a good agreement regarding treatment was presented, comparable to our study [31]. The variability in grading plus disease is concerning as it can determine whether a child receives treatment. This may lead experts to over-diagnose plus disease to meet the treatment criteria for Type 1. Conversely, if the camera applies pressure to the eye during imaging, the retinal vessels might misleadingly appear with less severe plus disease [19].

The level of agreement regarding ROP outcome analyzed with kappa statistics in this study was 'almost-perfect'. During our study, six patients fulfilled our criteria for receiving treatment, of whom three were due to Type 1 ROP and one due to A-ROP. In these cases, there was complete agreement regarding the indication for treatment. Lastly, two had late structural ROP complications, and disagreements regarding treatment occurred. Late structural changes due to ROP seen in children today are not described as part of the recommendations from the ETROP study conducted in 2004 and therefore disagreements among the graders were not surprising [22]. Treatment for ROP, outside the current

recommendations, have been reported and are often related to Type 1 in the fellow eye, logistical considerations, Stage 3 with pre-plus, and concerning late structural changes due to ROP [32,33]. Further, these patients often have a PMA > 40 weeks, which also was the case in our study. It is nonetheless reassuring that our telescreening setup showed a 100% feasibility within the current treatment recommendations and additionally managed to represent these cases.

4.5. Strengths and Limitations

We evaluated the feasibility of ROP screening by an NPHP but did not compare to BIO performed on the same patients. We decided against performing both fundus photography and BIO due to the vulnerable condition of the children as this would significantly increase the duration of the examination. Some challenges must be addressed in using widefield cameras in screening for ROP. Firstly, it may prolong the examination time when the NPHP is striving for the best frames to be evaluated by the ophthalmologist compared to BIO performed by an experienced ophthalmologist. Additionally, the patient's family must wait for the ophthalmologist to review the images and cannot be informed about the screening outcome immediately after the screening session. Lastly, a thorough education of healthcare professionals in ROP screening with a widefield camera is essential to ensure patient safety. However, these challenges are deemed manageable, and lessons learned from other telescreening setups could be applied. So far, wide-angled cameras have been evaluated as a reliable supplement for BIO, the gold-standard within ROP screening, but has not yet replaced it [9,28]. With the exponential development of technology, a potential benefit of including artificial intelligence in telescreening for ROP has already been investigated and would expectedly support decision-making in ROP in the nearest future [34]. Telescreening in ROP would be a benefit due to optimized resources within staff, evaluation of ROP in a stressless environment, avoiding unnecessary transfer of preterm babies and their families between hospitals for evaluation, and the possibility of tracking regression and progression in ROP.

Despite a high number of included screening sessions in this study, the number of patients receiving ROP treatment was low. The strength of this study is that all ROP treatments were performed in the same center in Denmark; accordingly, all patients fulfilling our treatment criteria for Type 1 ROP were included.

5. Conclusions

Telescreening with the ICON GO® camera performed by an NPHP showed an 'almost-perfect' agreement regarding screening outcome and the need for re-examinations was negligible. A standardized education and certification for imaging ROP should be setup for healthcare professionals to ensure patient safety. This setup can open up possibilities for implementing telemedicine on a national level in Denmark.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board at the department of Ophthalmology—Rigshospitalet and Danish Data Protection Agency (protocol code P-2020-1206, 18th of December 2020). The Danish National Medical Research Ethics Committee (VMK) waived the need for ethical board approval (decision number VMK-protokol_200921_V1, 3rd of February 2022).

Informed Consent Statement: Patient consent was waived due to a decision from The Danish National Medical Research Ethics Committee. ROP screening with widefield camera is a routine part of standard care for all preterm infants meeting national screening criteria. There was no additional risk to patients beyond their usual clinical care.

Data Availability Statement: The fundus photographs generated and analyzed during the current study are not publicly available due to privacy concerns. As the images are identifiable and may reveal the identity of individual patients, they cannot be shared to protect patient confidentiality in accordance with ethical standards. The raw data supporting the conclusions of this article can be made available by the corresponding author on request.

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References

1. International Committee for the Classification of Retinopathy of Prematurity (ICROP). An international classification of retinopathy of prematurity. Prepared by an international committee. *Br. J. Ophthalmol.* **1984**, *68*, 690–697. [CrossRef] [PubMed]
2. International Committee for the Classification of Retinopathy of Prematurity (ICROP). The international classification of retinopathy of prematurity revisited: An international committee for the classification of retinopathy of prematurity. *Arch. Ophthalmol.* **2005**, *123*, 991–999. [CrossRef] [PubMed]
3. International Committee for the Classification of Retinopathy of Prematurity (ICROP). International Classification of Retinopathy of Prematurity, Third Edition. *Ophthalmology* **2021**, *128*, e51–e68. [CrossRef]
4. Smith, L.E.H. Pathogenesis of retinopathy of prematurity. *Semin. Neonatol.* **2003**, *8*, 469–473. [CrossRef]
5. Fledelius, H.C.; La Cour, M. Behandlingskrævende præmaturitetsretinopati i Danmark (Danish). *J. Dan. Med. Assoc.* **2004**, *166*, 4360–4362. Available online: https://ugeskriftet.dk/nyhed/behandlingskraevende-praematuritetsretinopati-i-danmark (accessed on 2 September 2024).
6. Li, J.-P.O.; Liu, H.; Ting, D.S.J.; Jeon, S.; Chan, R.V.P.; Kim, J.E.; Sim, D.A.; Thomas, P.B.M.; Lin, H.; Chen, Y.; et al. Digital technology, tele-medicine and artificial intelligence in ophthalmology: A global perspective. *Prog. Retin. Eye Res.* **2021**, *82*, 100900. [CrossRef]
7. Jackson, K.M.; Scott, K.E.; Zivin, J.G.; Bateman, D.A.; Flynn, J.T.; Keenan, J.D.; Chiang, M.F. Cost-Utility Analysis of Telemedicine and Ophthalmoscopy for Retinopathy of Prematurity Management. *Arch. Ophthalmol.* **2008**, *126*, 493–499. [CrossRef]
8. Trese, M.T. What is the real gold standard for ROP screening? *Retina* **2008**, *28*, S1–S2. [CrossRef]
9. Dhaliwal, C.; Wright, E.; Graham, C.; McIntosh, N.; Fleck, B.W. Wide-field digital retinal imaging versus binocular indirect ophthalmoscopy for retinopathy of prematurity screening: A two-observer prospective, randomised comparison. *Br. J. Ophthalmol.* **2009**, *93*, 355–359. [CrossRef]
10. Brady, C.J.; D'amico, S.; Campbell, J.P. Telemedicine for Retinopathy of Prematurity. *Telemed. e-Health* **2020**, *26*, 556–564. [CrossRef]
11. Quinn, G.E.; Ying, G.-S.; Daniel, E.; Hildebrand, P.L.; Ells, A.; Baumritter, A.; Kemper, A.R.; Schron, E.B.; Wade, K.; e-ROP Cooperative Group. Validity of a Telemedicine System for the Evaluation of Acute-Phase Retinopathy of Prematurity. *JAMA Ophthalmol.* **2014**, *132*, 1178–1184. [CrossRef] [PubMed]
12. Morrison, D.; Bothun, E.D.; Ying, G.-S.; Daniel, E.; Baumritter, A.; Quinn, G. Impact of number and quality of retinal images in a telemedicine screening program for ROP: Results from the e-ROP study. *J. Am. Assoc. Pediatr. Ophthalmol. Strabismus* **2016**, *20*, 481–485. [CrossRef]
13. Simkin, S.K.; Misra, S.L.; Han, J.V.; McGhee, C.N.J.; Dai, S. Auckland regional telemedicine retinopathy of prematurity screening network: A 10-year review. *Clin. Exp. Ophthalmol.* **2019**, *47*, 1122–1130. [CrossRef] [PubMed]
14. Ho, T.; Lee, T.C.; Choe, J.Y.; Nallasamy, S. Evaluation of real-time video from the digital indirect ophthalmoscope for telemedicine consultations in retinopathy of prematurity. *J. Telemed. Telecare* **2020**, *28*, 502–507. [CrossRef]
15. Campbell, J.P.; Ryan, M.C.; Lore, E.; Tian, P.; Ostmo, S.; Jonas, K.; Chan, R.P.; Chiang, M.F. Diagnostic Discrepancies in Retinopathy of Prematurity Classification. *Ophthalmology* **2016**, *123*, 1795–1801. [CrossRef]
16. Greenwald, M.F.; Danford, I.D.; Shahrawat, M.; Ostmo, S.; Brown, J.; Kalpathy-Cramer, J.; Bradshaw, K.; Schelonka, R.; Cohen, H.S.; Chan, R.V.P.; et al. Evaluation of artificial intelligence-based telemedicine screening for retinopathy of prematurity. *J. AAPOS* **2020**, *24*, 160–162. [CrossRef]

17. Le Nguyen, T.A.; Raileanu, V.; Smith, B.; Griffin, A.; Shah, S. Excellent accuracy of trained neonatal nurses in the detection of referral-warranted retinopathy of prematurity within an established telemedicine screening programme. *J. Paediatr. Child Health* **2024**, *60*, 369–374. [CrossRef]
18. Athikarisamy, S.E.; Lam, G.C.; Ross, S.; Rao, S.C.; Chiffings, D.; Simmer, K.; Bulsara, M.K.; Patole, S. Comparison of wide field imaging by nurses with indirect ophthalmoscopy by ophthalmologists for retinopathy of prematurity: A diagnostic accuracy study. *BMJ Open* **2020**, *10*, e036483. [CrossRef]
19. Tan, Z.; Isaacs, M.; Zhu, Z.; Simkin, S.; He, M.; Dai, S. Retinopathy of prematurity screening: A narrative review of current programs, teleophthalmology, and diagnostic support systems. *Saudi J. Ophthalmol.* **2022**, *36*, 283–295. [CrossRef]
20. Fan, K.C.; Read, S.P.; Patel, N.A.; Vanner, E.A.; Al-Khersan, H.; Laura, D.M.; Pakravan, P.; Negron, C.I.; Berrocal, A.M. Fundus Pigmentation in the Diagnosis and Treatment of Retinopathy of Prematurity. *Ophthalmology* **2021**, *128*, 1242–1244. [CrossRef]
21. Chan, R.V.P.; Chiang, M.F.; Jonas, K. Retinopathy of Prematurity: Case-Based Training. Available online: https://www.aao.org/education/interactive-tool/retinopathy-of-prematurity-case-based-training (accessed on 2 September 2024).
22. Good, W.V. Final results of the early treatment for retinopathy of prematurity (ETROP) randomized trial. *Trans. Am. Ophthalmol. Soc.* **2004**, *102*, 233–248. [PubMed] [PubMed Central]
23. Fierson, W.M. Screening examination of premature infants for retinopathy of prematurity. *Pediatrics* **2018**, *142*, e20183061. [CrossRef] [PubMed]
24. Sanghi, G.; Dogra, M.R.; Dogra, M.; Katoch, D.; Gupta, A. A hybrid form of retinopathy of prematurity. *Br. J. Ophthalmol.* **2012**, *96*, 519–522. [CrossRef]
25. Landis, J.R.; Koch, G.G. The Measurement of Observer Agreement for Categorical Data. *Biometrics* **1977**, *33*, 159–174. [CrossRef]
26. Kirkwood, B.R.; Sterne, J.A.C. *Essential Medical Statistics*, 2nd ed.; Blackwell Publishing Ltd.: Oxford, UK, 2003.
27. e-ROP Cooperative Group. Training retinal imagers for retinopathy of prematurity (ROP) screening. *Physiol. Behav.* **2017**, *176*, 139–148. [CrossRef]
28. Maka, E.; Kovács, G.; Imre, L.; Gilbert, C.; Szabó, M.; Németh, J.; Nagy, Z.Z.; Somogyvári, Z. The validity of telemedicine-based screening for retinopathy of prematurity in the Premature Eye Rescue Program in Hungary. *J. Telemed. Telecare* **2021**, *27*, 367–375. [CrossRef]
29. Pasricha, M.V.; Ludwig, C.A.; Moshfeghi, D.M. Stanford University Network for Diagnosis of Retinopathy of Prematurity (SUNDROP): Truly Mobile Teleophthalmology. *Ophthalmic Surg Lasers Imaging Retin.* **2021**, *52*, 11–12. [CrossRef]
30. Wang, S.K.; Korot, E.; Zaidi, M.; Ji, M.H.; Al-Moujahed, A.; Callaway, N.F.; Kumm, J.; Moshfeghi, D.M. Modeling absolute zone size in retinopathy of prematurity in relation to axial length. *Sci. Rep.* **2022**, *12*, 4717. [CrossRef]
31. Slidsborg, C.; Forman, J.L.; Fielder, A.R.; Crafoord, S.; Baggesen, K.; Bangsgaard, R.; Fledelius, H.C.; Greisen, G.; la Cour, M. Experts do not agree when to treat retinopathy of prematurity based on plus disease. *Br. J. Ophthalmol.* **2012**, *96*, 549–553. [CrossRef]
32. Lemaître, D.; Barjol, A.; Abdelmassih, Y.; Farnoux, C.; Martin, G.C.; Metge, F.; Chapron, T.; Caputo, G. Treatment outside the recommended guidelines for retinopathy of prematurity (Rop): Prevalence, characteristics, and issues. *J. Clin. Med.* **2022**, *11*, 39. [CrossRef]
33. Liu, T.; Tomlinson, L.A.; Ying, G.S.; Yang, M.B.; Binenbaum, G. Treatment of non–type 1 retinopathy of prematurity in the Postnatal Growth and Retinopathy of Prematurity (G-ROP) study. *J. AAPOS* **2019**, *23*, 332.e1–332.e6. [CrossRef] [PubMed]
34. Coyner, A.S.; Murickan, T.; Oh, M.A.; Young, B.K.; Ostmo, S.R.; Singh, P.; Chan, R.V.P.; Moshfeghi, D.M.; Shah, P.K.; Venkatapathy, N.; et al. Multinational External Validation of Autonomous Retinopathy of Prematurity Screening. *JAMA Ophthalmol.* **2024**, *142*, 327–335. [CrossRef] [PubMed]

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Review

Ocular Ultrasound in the Diagnosis of Optic Neuropathies: A Review of the Literature

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Abstract: Background: Optic neuropathies represent one of the most frequent causes of vision loss, and they can manifest alone or in conjunction with neurological or systemic symptoms and signs. In recent years, the diagnostic techniques used to detect optic neuropathies have significantly improved, facilitating diagnosis and improving treatment. Among these, ocular ultrasound has assumed a fundamental role, although with conflicting results in the published scientific literature. For this reason, the aim of this review is to analyze the role of ocular ultrasound in the precise and targeted diagnosis of optic neuropathies to better understand the presumed potential of this precious diagnostic tool in the management of these ocular and neurological disorders. Methods: We carried out a search on PubMed and Scopus utilizing terms related to optic neuropathies and ocular ultrasound, including only relevant English full-length research articles, case reports, or case series. Results: Most of the papers published in the scientific literature use only the B-scan ultrasound technique without considering the more precise and objective standardized A-scan technique that allows for performing more accurate diagnostic tests, such as the "30-degree test" and the "optic nerve exercise test". Conclusions: Future clinical trials and research on optic neuropathies should also consider the use of the standardized A-scan technique in order to compare clinical findings not only with B-scan ultrasonography but also with other noninvasive procedures that could be helpful in reaching the correct diagnosis.

Keywords: glaucoma; ocular ultrasound; optic nerve; optic neuritis; optic neuropathies

1. Introduction

An isolated occurrence of vision loss associated with a more general neurological condition can be caused by disorders of the optic nerve, also known as optic neuropathies. There are many different neurological diseases for which optic neuropathy is frequently the presenting sign, with varied different etiologies [1].

Optic neuropathy is the term used to describe the malfunction and/or degeneration of the optic nerve's axons, which leads to optic nerve atrophy. Axonal injury and death of retinal ganglion cells are typical features of several optic neuropathies [2] (Table 1).

The two main degenerative causes of damage to the optic nerve are glaucoma and optic neuritis [2].

The site of the damage causing the visual loss is the only thing determined by the diagnosis of the optic neuropathy [3,4]. An optic neuropathy cannot be regarded as a disease unto itself; rather, physicians must first categorize the condition based on the assumed mechanism of the optic neuropathy before trying to use ancillary tests to determine the precise etiology of this pathological condition [5,6]. Understanding the impairments that commonly indicate optic neuropathy and the traits of the various causes aids clinicians in reducing a broad differential diagnosis and carrying out a successful diagnostic evaluation [7].

Table 1. Differential diagnosis of optic neuropathy.

Glaucoma
Inflammatory
- Idiopathic inflammatory optic neuritis (often associated with multiple sclerosis)
- Neuromyelitis optica
- Systemic inflammatory and autoimmune diseases
- Infectious diseases
Vascular
- Anterior or posterior
- Arteritic or non-arteritic
- Post-radiation therapy
Compressive or infiltrative
- Neoplastic
- Non-neoplastic
Paraneoplastic
Toxic
Nutritional
Hereditary
Traumatic

Ultrasonographic assessment of the optic nerve sheath diameter (ONSD) has been a popular tool for optic nerve examination in recent years [8–10]. In addition, ultrasonography is a quick, affordable, safe, non-invasive diagnostic technique that may be quite helpful for bedside evaluations of critically ill patients. However, a well-trained and skilled operator is needed to perform a reliable and well-executed ocular ultrasonography for optic nerve assessment, and several precautions should be taken in the exam execution as well as in the employed probe and technique to obtain trustworthy results [11–14].

For this reason, the aim of this review is primarily to illustrate the technique of performing ocular ultrasound for the evaluation of the optic nerve and secondarily to analyze the published scientific literature to highlight the potential role of ocular ultrasound in the evaluation of the optic nerve in cases of different optic neuropathies.

2. Materials and Methods

We utilized two medical databases, PubMed and Scopus, to perform our search. A preliminary general Google search was also performed in order to have a wider viewpoint. Terms like "ocular ultrasonography", "point-of-care ultrasound", "POCUS", "optic neuropathies", "glaucoma", and "optic neuritis" were entered in. The search sentences that were included in the text were either chosen straight from relevant bibliographies or with consideration for the body of current literature. Manual searches of the bibliographies were also carried out in order to find more inclusions. Only English full-length research articles, case reports, or case series pertaining to the ultrasonography assessment of the optic nerve in cases of optic neuropathies were included in this review.

3. Ultrasound Technique

Typically, a high-frequency and high-resolution (5–14 MHz) linear array probe is used for B-scan ultrasonography examinations. Generally, the patients are assessed while lying supine, with the head raised approximately 30–40 degrees.

Most clinicians gently apply a standard ultrasonic gel to the closed eyelid and orient the probe at the right angle to observe the optic nerve insertion.

Nevertheless, as the patient's gaze direction is not visible, this might result in mistakes in the evaluation and measurement of the ONSD and optic nerve. Considering this, skilled ophthalmologists typically perform this diagnostic exam with open lids and with the use of methylcellulose and anesthetic drops, increasing examination accuracy [15,16].

The optic disc is utilized as a reference point for measuring the ONSD from inner edge to inner edge at a distance of 3 mm behind the globe after adjusting the B-scan probe to better view the optic nerve insertion.

Apart from the B-scan examination, "standardized echography" is another well-known ultrasound method in the field of ophthalmology. It is often used with the ultrasonic probe in direct contact with the surface of the eye, combining the A, B, and Doppler methods [15]. This diagnostic method's uniqueness is directly related to the A-scan instrumentation's unique design and standardization. In fact, when analyzing the same structure, standardization enables each examiner to acquire the same echograms, resulting in similar and reproducible findings [15]. Moreover, a patient's age or overall health do not restrict the use of the standardized ultrasonography. However, sedation may be necessary for babies aged six months to three years in order to facilitate a comprehensive and exhaustive examination, while it is performed using only anesthetic drops in adults [15]. Standardized echography can measure the thickness of the optic nerve with an accuracy of +0.3 mm in the retrobulbar space and +0.5 mm in the posterior orbit [15]. Additionally, this ultrasonography method may identify pathological conditions, including meningioma and glioma, as well as neural swelling, increased thickness of the optic nerve sheaths, increased subarachnoid fluid, atrophy, and other disorders [15,17].

Regretfully, B-scan ultrasonography should not be considered an especially objective method of assessing the optic nerve. In fact, when evaluating small anatomical structures, such as the optic nerve, this ultrasound technique, which has been previously evaluated in the literature [18,19], has a number of drawbacks since the B-mode does not have a uniformity of the gain setting [20,21], also known as the "blooming effect" [15,22]. Consequently, this pitfall may be resolved by the standardized A-scan methodology, a blooming effect-free approach that employs an 8 MHz non-focused probe with a customized S-shaped amplification and enables more precise measurements, particularly in the case of the optic nerve assessment [15–23].

This method can also avoid the problem of calipers being positioned incorrectly and yield more accurate and exact findings by clearly showing strong reflecting spikes from the interface between arachnoid and subarachnoid fluid [15].

In addition, the standardized A-scan examination may be used to perform the "30-degree test" [11]. This test allows for clinicians to differentiate between an increase in ONSD caused by raised intracranial pressure related to increased subarachnoid fluid and an increase in ONSD caused by other disorders, such as optic neuritis or optic nerve meningioma. This maneuver is carried out with the patient looking straight ahead and then to the lateral side in healthy and cooperative patients. If this test demonstrates a decrease in the maximal diameter of at least 5%, it will demonstrate intracranial hypertension caused by increased subarachnoidal fluid, which causes ONSD distension; on the other hand, a solid lesion of the optic nerve or its inflammatory process will not lead to this reduction [11,15] (Figure 1).

Finally, the "optic nerve exercise test" can be used to rule out optic nerve compartment syndrome. In this case, the patient will be asked to look for 15 to 20 s at the very right and left lateral sides in turn throughout this test. This three-minute test is followed by a two-minute eye closure to allow for the subarachnoid fluid—which was driven out of the orbit during the exercise—to return to the baseline position. In healthy people, the orbital subarachnoid fluid frequently returns to its baseline level, unlike what happens in the presence of optic nerve compartment syndrome [14].

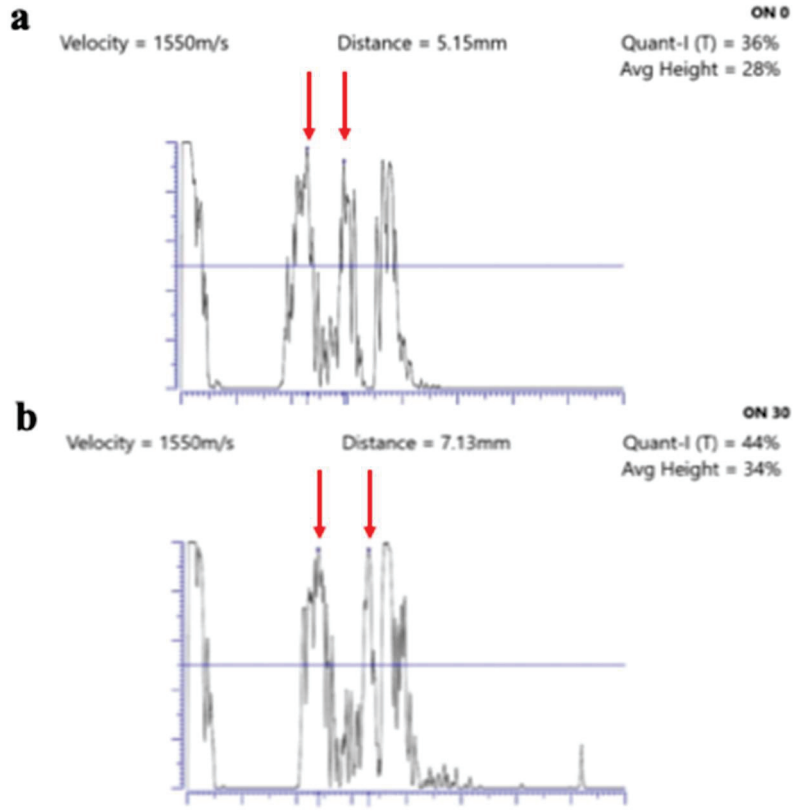

Figure 1. Standardized A-scan ultrasonography of a patient in the primary gaze position (**a**) with an enlargement of the optic nerve (5.15 mm). After the "30-degree test", (**b**) it is possible to observe the non-reduction in the distance between the hyperreflective peaks (red arrows—7.13 mm), thus demonstrating an increase in the thickness of the optic nerve not related to an increase in intracranial pressure.

4. Ocular Ultrasound in Glaucoma

Considering the previously described ultrasound technique [15], it can be used in the same way for the evaluation of the optic nerve in all the optic neuropathies. In particular, for the B-scan, the probe is placed in the temporal portion of the open eye in the primary gaze direction and is tilted until the optic nerve insertion is visualized; on the other hand, when the standardized A-scan technique is performed, the probe is always placed temporally, but this time, the peaks corresponding to the medial rectus muscle are first visualized using it as a reference point. Subsequently, the probe is tilted to identify the peaks corresponding to the optic nerve so that its measurement can be carried out [15]. However, as previously stated, the standardized A-scan technique requires the operator to be skilled in this type of diagnostic evaluation [16–23].

One of the main and most widespread diseases of the optic nerve is certainly glaucoma. Kerlen, in the 1980s, already stated how the optic nerve of glaucomatous patients could be evaluated through excavation of the disc with B-scan ultrasonography, even with media opacities [24]. Conversely, the use of ocular ultrasound in glaucomatous patients to evaluate any other alterations affecting the optic nerve is quite controversial, considering that there are also few published papers on this challenging topic [25–28].

Abegão Pinto and colleagues [25] were the first to investigate with B-scan ultrasound how ONSD differs between glaucomatous patients and healthy people. They enrolled 46 patients with normal-tension glaucoma (NTG) (patients with optic disc damage and visual field loss), 61 patients with primary open-angle glaucoma (POAG) (patients with untreated intraocular pressure of 21 mmHg or above), and 42 healthy control subjects. They found that ONSD did not significantly differ between healthy, NTG, and POAG patients, with no correlation between visual field damage and ONSD in either of the glaucoma

groups. On the other hand, ONSD was found to be correlated with intraocular pressure only in NTG patients. For this reason, the authors hypothesized that the translaminar pressure gradient may play a special role in this particular form of glaucoma [25].

Willekens et al. [26] tried to determine the influence of retrobulbar hemodynamics on ONSD values in glaucomatous patients evaluated by ultrasound color Doppler imaging. In particular, they assessed 88 POAG patients, 58 NTG patients, and 51 healthy controls, and they did not find any significant differences in ONSD between the three study groups, while ONSD was found to be negatively correlated with retrobulbar blood flow velocities in glaucomatous patients but not in healthy controls.

In 2018, Liu et al. [27] studied the optic nerve with ultrasound in 40 NTG patients and 42 POAG patients, comparing them with 46 healthy controls, analyzing the effect that low cerebrospinal fluid pressure in the subarachnoid space of the retrobulbar optic nerve (ONSASA) has on the trans-lamina cribrosa pressure difference in NTG. The results showed that ONSASA was significantly smaller in the NTG group compared to the POAG or control groups, confirming the importance that cerebrospinal fluid pressure has on the trans-lamina cribrosa pressure difference in the NTG etiopathogenesis.

Finally, Omatiga and co-authors [28] evaluated 60 glaucomatous adults and 60 healthy age- and sex-matched individuals to sonographically assess potential changes in the ONSD between the two groups, finding the ONSD of the glaucomatous eyes to be substantially thinner compared to the matching control eyes.

In conclusion, considering the conflicting results and the presence of few clinical studies, further studies are needed to better understand the potential role of the ultrasound ONSD and ocular ultrasonography in the diagnosis and management of glaucoma.

5. Ocular Ultrasound in Acute Optic Neuritis

The usefulness of ocular ultrasound in evaluating the optic nerve in cases of inflammatory processes, such as optic neuritis, has been known since the 1980s [15,24,29]. In fact, with B-scan ultrasonography, it is also possible to appreciate the presence of papilledema at the optic nerve insertion, with an elevation of the optic disc (Figure 2).

Several published papers already analyzed the role of ocular ultrasonography in the diagnosis and management of acute optic neuritis.

In the 1990s, a great emphasis was placed on the analysis of the optic nerve with the standardized A-scan method, and Dees et al. [30] performed a pilot study of 27 patients by performing B-scan and standardized A-scan ultrasound on patients presenting with optic neuritis. The 30-degree test was considered to be positive if the swelling decreased by at least 10% on abduction. They classified three groups of patients according to their ultrasound characteristics: normal nerves, swollen nerves with a positive 30-degree test, and swollen nerves with a negative 30-degree test. The results showed a substantial increase in optic nerve diameter in 74% of cases and a positive correlation between nerve swelling and the severity of initial visual loss.

During the same years, Elvin et al. [31] emphasized the importance of Doppler ultrasonography in optic neuritis to determine nerve morphology, nerve swelling, and resistance to flow in the central retinal artery, correlating them with magnetic resonance imaging and visual evoked potentials. The ultrasound examinations were performed on a dynamic scanner with a 7.5 MHz linear transducer within a mean of 27 days of the onset of symptoms. A statistically significant difference was found in the optic nerve diameter and in the resistance to flow in the central retinal artery between the affected and unaffected eyes, confirming the possibility of using Doppler as an indicator of optic nerve inflammation activity.

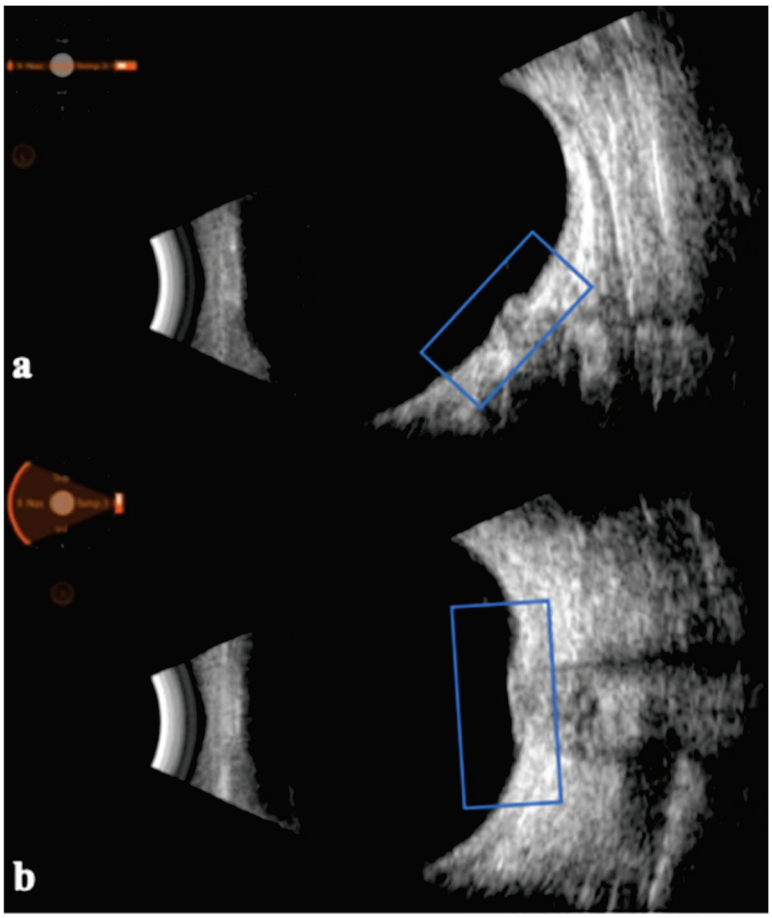

Figure 2. B-scan ultrasonography of the optic nerve insertion in the sagittal (**a**) and transversal (**b**) planes in a patient with papilledema. It is possible to observe the prominence of the optic disc (blue box).

The possibility of increasing the quality of diagnosis with Doppler ultrasound of the optic nerve was also taken up some years later by Karaali et al. [32] and Karami et al. [33].

The first ones evaluated orbital blood flow velocities with Doppler sonography in 20 patients with acute unilateral optic neuritis, and they found that peak systolic and end diastolic velocities in the ophthalmic artery were significantly increased in the affected orbits.

On the other hand, Karami et al. [33] subverted the conception of Doppler ultrasound for studying the optic nerve because, contrary to the previous studies [31,32], they showed that there were no significant differences in orbital blood flow parameters between the eye with optic neuritis and the healthy contralateral eye of the 23 enrolled patients.

Lochner and colleagues [34–36] also utilized B-scan ultrasonography to evaluate the possibility to measure optic nerve diameter and ONSD in patients affected by unilateral optic neuritis, comparing them with healthy controls. They found a statistically significant difference between the two groups in all their clinical research, with a significant thickening of the ONSD in the patients affected by optic neuritis.

In addition, several case reports and case series analyzed the role of ocular ultrasound in detecting optic neuritis in the emergency department [37–39].

Wayman and Carmody [37] assessed a 37-year-old diabetic patient complaining of visual loss in the left eye, initially without pain and then with a feeling of pressure on the eye. He underwent a B-scan ultrasonography, which revealed an ONSD dilatation of 6.21 mm and an elevation of the optic disc, both consistent with papilledema or an optic disc swelling. The diagnosis of optic neuritis was then confirmed following an ophthalmological and neurological consultation, also performing a brain magnetic resonance imaging.

Similarly, in two other case series [38,39], the authors were able to make a diagnosis of optic neuritis thanks to the patient's symptoms and clinical signs and by carrying out

an ocular ultrasound with the B-scan method, which revealed a notable thickening of the affected ONSD compared to the healthy contralateral eye.

6. Ocular Ultrasound in Optic Neuritis Related to Demyelinating Disorders

Optic neuritis, particularly retrobulbar optic neuritis, can also represent the initial symptom of a central nervous system disease: in particular, demyelinating disorders such as multiple sclerosis [40].

The role of optic nerve ultrasound examination in evaluating retrobulbar optic neuritis was studied in 2005 by Titlić et al. [41] and in 2010 by Stefanović et al. [42].

Titlić et al. [41] prospectively evaluated 20 patients with multiple sclerosis and compared the results with those obtained by magnetic resonance imaging. They found that the diameter of the optic nerve with retrobulbar neuritis showed statistically significant differences from the healthy one and correlated significantly with the number of multiple sclerosis brain lesions.

Stefanović et al. [42], instead, used the B-scan method in 23 patients presenting with retrobulbar optic neuritis and papillitis. Similar to the study performed by Dees et al. [30], they also performed the 30-degree test during the optic nerve assessment. The results showed that the retrobulbar region of the optic nerve was thicker in 94% of the patients with retrobulbar neuritis and in all the patients with papillitis, also finding a correlation between the reduction in visual acuity and the thickening of the retrobulbar region of the optic nerve.

Raeesmohammadi and colleagues [43] sonographically evaluated 60 patients affected by multiple sclerosis who had not recently received an optic neuritis diagnosis, comparing them with 60 healthy sex- and age-matched volunteers. They found that, when optic nerve diameter and ONSD were evaluated by ocular ultrasound, the patients affected by multiple sclerosis who were not currently experiencing an optic neuritis had considerably lower levels than their healthy controls, indicating a gradual subclinical axonal loss.

Similar results were also detected by De Masi et al. [44] and Schroeder et al. [45] who found optic nerve diameter and ONSD evaluated with ocular ultrasound were smaller in patients affected by multiple sclerosis compared to healthy controls, suggesting a chronic depletion of axons in this demyelinating disorder.

Conversely, Elkholy et al. [46] assessed 25 patients with a first attack of an acute demyelinating optic neuritis compared to 25 healthy subjects using ocular ultrasound, showing a significant thickening of ONSD compared to the controls. Furthermore, they also found the ONSD thickness and visual acuity to be significantly inversely correlated.

An increased ultrasound ONSD in the case of patients with optic neuritis related to multiple sclerosis was also found by Saigh et al. [47] and Kwon et al. [48], while Koraysha and colleagues [49] found no significant differences of optic nerve diameter and ONSD between the patients' group and control group.

In addition, Krogias et al. [50] evaluated 34 optic nerves from 17 patients affected by chronic inflammatory demyelinating polyradiculoneuropathy compared to 30 optic nerves from 15 healthy subjects. Optic nerve diameter and ONSD were sonographically measured, with no significant differences between the two study groups.

Finally, Candeliere Merlicco and co-authors [51] aimed to evaluate the utility of transorbital ultrasonography in the quantification of optic nerve atrophy in multiple sclerosis patients and to determine whether optic nerve atrophy correlates with the disease duration and disability. They enrolled 59 patients diagnosed with relapsing–remitting multiple sclerosis and 36 healthy controls. The ultrasound ONSD was found to be smaller in the patients with multiple sclerosis and to be correlated with the Expanded Disability Status Scale and with the duration of the disease without being interfered by the previous history of optic neuritis.

In conclusion, in cases of demyelinating diseases, the results present in the literature are conflicting, as the thickness of the ONSD measured with ocular ultrasound is variable based on the state of the disease and the presence of acute inflammation of the optic

nerve. In fact, in the presence of an acute inflammatory process, the ONSD is increased in thickness, while in the presence of a demyelinating disorder alone, the ONSD is reduced in size compared to healthy controls.

The main findings published in the scientific literature related to optic neuritis are summarized in Table 2.

Table 2. Summary of the studies analyzing the role of ocular ultrasound in optic neuritis.

Authors (Year)	Ref.	N. Patients	Ultrasound Technique	Outcomes
Dees et al. (1995)	[30]	27 patients with idiopathic optic neuritis	B-scan and standardized A-scan techniques	Substantial increase in optic nerve diameter in 74% of cases and a positive correlation between nerve swelling and the severity of initial visual loss.
Elvin et al. (1998)	[31]	18 patients with a clinical diagnosis of optic neuritis	Doppler ultrasonography	A statistically significant difference was found in the optic nerve diameter and in the resistance to flow in the central retinal artery between the affected and unaffected eyes, confirming the possibility of using Doppler as an indicator of optic nerve inflammation activity.
Karaali et al. (2003)	[32]	20 patients with acute unilateral optic neuritis	Doppler ultrasonography	Peak systolic and end diastolic velocities in the ophthalmic artery were significantly increased in the affected eyes.
Karami et al. (2012)	[33]	23 patients with acute unilateral optic neuritis	Doppler ultrasonography	No significant differences were found in orbital blood flow parameters between the eye with optic neuritis and the healthy contralateral eye.
Lochner et al. (2017)	[34]	23 patients with unilateral optic neuritis and 19 sex- and age-matched healthy controls	B-scan ultrasonography	Optic nerve sheath diameter and optic nerve diameter are increased in the affected eye.
Lochner et al. (2014)	[35]	21 patients with unilateral optic neuritis and 21 sex- and age-matched healthy controls	B-scan ultrasonography	The median optic nerve sheath diameter and optic nerve diameter were thicker on the affected side compared with the nonaffected side and controls.
Lochner et al. (2017)	[36]	45 patients with newly diagnosed optic neuritis	B-scan ultrasonography	The median optic nerve sheath diameter was thicker on the affected side compared with the nonaffected side.
Wayman and Carmody (2014)	[37]	37-year-old diabetic patient complaining of visual loss in the left eye	B-scan ultrasonography	Optic nerve sheath diameter was found to be 6.21 mm with an elevation of the optic disc. The diagnosis of optic neuritis was then confirmed following an ophthalmological and neurological consultation, also performing a brain magnetic resonance imaging.
Badron and Ong (2019)	[38]	15-year-old girl with optic neuritis	B-scan ultrasonography	Transorbital ultrasound revealed an irregularly enlarged optic nerve sheath with increased optic nerve sheath diameter (5.1 mm) and an elevated optic disc height (0.5 mm). The ultrasound findings correlated well with her magnetic resonance imaging of her orbit.
Yee et al. (2019)	[39]	A 35-year-old man, a 48-year-old man, a 34-year-old female, and a 28-year-old female with optic neuritis	B-scan ultrasonography	Optic nerve sheath diameters of the affected eyes were all found to be more than 5.5 mm.
Titlić et al. (2005)	[41]	20 patients with multiple sclerosis and retrobulbar neuritis	B-scan ultrasonography	The diameter of the optic nerve with retrobulbar neuritis showed statistically significant differences from the healthy one and correlated significantly with the number of multiple sclerosis brain lesions.
Stefanović et al. (2010)	[42]	23 patients presenting with retrobulbar optic neuritis and papillitis	B-scan and standardized A-scan techniques	The retrobulbar region of the optic nerve was found to be thicker in 94% of the patients with retrobulbar neuritis and in all the patients with papillitis, also finding a correlation between the reduction in visual acuity and the thickening of the retrobulbar region of the optic nerve.
Raeesmohammadi et al. (2020)	[43]	60 patients affected by multiple sclerosis who had not recently received an optic neuritis diagnosis, and 60 healthy sex- and age-matched volunteers.	B-scan ultrasonography	Patients affected by multiple sclerosis who were not currently experiencing an optic neuritis had considerably lower levels of optic nerve sheath diameter and optic nerve diameter than their healthy controls, indicating a gradual subclinical axonal loss.
De Masi et al. (2016)	[44]	60 unselected relapse-free multiple sclerosis patients and 35 matched healthy controls	B-scan ultrasonography	Optic nerve diameter and optic nerve sheath diameter evaluated with ocular ultrasound were found to be smaller in patients affected by multiple sclerosis compared to healthy controls, suggesting a chronic depletion of axons.

Table 2. *Cont.*

Authors (Year)	Ref.	N. Patients	Ultrasound Technique	Outcomes
Schroeder et al. (2020)	[45]	78 patients with a history of demyelinating disorders	B-scan ultrasonography	Optic nerve diameter and optic nerve sheath diameter evaluated with ocular ultrasound were found to be smaller in patients affected by multiple sclerosis compared to healthy controls, suggesting a chronic depletion of axons.
Elkholy et al. (2020)	[46]	25 patients with a first attack of an acute demyelinating optic neuritis compared to 25 healthy subjects	B-scan ultrasonography	A significant thickening of optic nerve sheath diameter was found compared to controls. Furthermore, optic nerve sheath diameter thickness and visual acuity were found to be significantly inversely correlated.
Saigh et al. (2019)	[47]	59-year-old female patient with progressive unilateral visual loss while she was receiving outpatient treatment for relapsing–remitting multiple sclerosis	B-scan ultrasonography	Transorbital ultrasound revealed a disparity between the optic nerve sheath diameters of the affected and nonaffected eyes and striking optic nerve edema in the affected eye, thus diagnosing an acute optic neuritis.
Kwon et al. (2019)	[48]	17 patients with first-attack unilateral acute optic neuritis	B-scan ultrasonography	Ocular ultrasonography revealed thickening of the optic nerve diameter and optic nerve sheath diameter on the affected side compared with the unaffected side.
Koraysha et al. (2019)	[49]	23 patients affected by relapsing–remitting multiple sclerosis with a history of optic neuritis, 26 patients affected by relapsing–remitting multiple sclerosis without a history of optic neuritis, and 50 age- and sex-matched healthy controls	B-scan ultrasonography	No significant differences of optic nerve diameter and optic nerve sheath diameter were found between the patients' group and control group.
Krogias et al. (2016)	[50]	17 patients affected by chronic inflammatory demyelinating polyradiculoneuropathy compared to 15 healthy subjects	B-scan ultrasonography	Optic nerve diameter and optic nerve sheath diameter showed no significant differences between the two study groups.
Candeliere Merlicco et al. (2018)	[51]	59 patients diagnosed with relapsing–remitting multiple sclerosis and 36 healthy controls	B-scan ultrasonography	The ultrasound optic nerve sheath diameter was found to be smaller in patients with multiple sclerosis and to be correlated with the Expanded Disability Status Scale and with the duration of the disease without being interfered by the previous history of optic neuritis.

7. Ocular Ultrasound in Other Optic Neuropathies

In 1997, Gerling et al. [52] used ultrasound to study optic nerve diameter in idiopathic optic neuritis and anterior ischemic optic neuropathy. They evaluated 25 patients, 16 with idiopathic optic neuritis and 9 with anterior ischemic optic neuropathy, with a B-scan ultrasound (10 MHz); the probe was placed on the closed upper eyelid while the patient looked temporally. The optic nerve diameter in the patients with optic neuritis with and without disc swelling was significantly larger than that in the contralateral healthy eye. This difference was not confirmed in the patients with anterior ischemic optic neuropathy, and this can be explained by the fact that, in the patients with idiopathic optic neuritis with and without disc swelling, the enlargement of the optic disc is probably caused by an inflammatory edema, which is not present in the case of a vascular optic neuropathy.

Similarly, Dehghani and colleagues [53] evaluated the usefulness of B-scan ultrasonography in distinguishing optic neuritis from non-arteritic anterior ischemic optic neuropathy by measuring the diameter of the optic nerve. They compared 10 consecutive patients with optic neuritis, 10 patients with non-arteritic anterior ischemic optic neuropathy, 10 young controls, and 10 elderly controls. The diameter of the optic nerve was measured using B-scan ultrasonography (13 MHz) by a single radiologist who was not aware of the diagnosis or the side of the lesion. In the optic neuritis patients, the mean diameter of the affected nerve was significantly larger than that of the unaffected nerve and also larger than that of the healthy controls. On the other hand, in the non-arteritic anterior ischemic optic neuropathy patients, there was no significant difference between the mean diameter of the affected nerves and the control group. Moreover, the diameter of the affected nerve was significantly larger in the optic neuritis patients than in the non-arteritic anterior ischemic optic neuropathy patients. For this reason, the authors concluded that B-scan ultrasonography is helpful in the early stages of optic neuropathy to distinguish

optic neuritis from non-arteritic anterior ischemic optic neuropathy in those cases for which the diagnosis is still uncertain after clinical evaluation.

Finally, Ji et al. [54] evaluated the ONSD in eyes with dysthyroid optic neuropathy (DON) and its relationship with clinical characteristics and disease severity. A total of 47 healthy eyes, 36 thyroid-associated ophthalmopathy (TAO) eyes without DON, and 33 eyes with DON were studied. All subjects underwent transbulbar B-scan ultrasonography performed by a single investigator using a 10 MHz probe. The authors demonstrated that ONSDs measured at 3 mm and 6 mm of DON eyes were significantly higher than those in non-DON eyes and healthy eyes, showing that ONSD can be potentially used to distinguish DON eyes from non-DON eyes, although the ultrasound ONSD characteristics of chronic DON eyes need to be further examined.

8. Discussion

Nowadays, optic neuropathy represents a very dangerous pathological disorder for vision as well as often being the presenting sign of more complex systemic diseases [1,2]. For this reason, a quick and timely diagnosis of this pathological condition could be crucial in order to best manage and treat this clinical entity, thus limiting complications.

This gap may be filled by using ultrasound measurement of ONSD, which has been demonstrated to be a safe, noninvasive bedside diagnostic method that allows for the early start of therapy and the real-time assessment of optic neuropathies. In this therapeutic context, it appears to have unrealized potential even though it is not a revolutionary method [15].

Currently, the biggest pitfall is the substantial variation in ultrasound ONSD reported in the clinical research, as evidenced by conflicting cut-off value results and morphological findings from the published scientific literature pertaining to various optic neuropathies, particularly optic neuritis and glaucoma. Since the B-scan is the diagnostic tool that clinicians use most frequently to assess optic nerves, it is possible that the primary cause of this problem is the lack of uniformity in this ultrasound method. In fact, as previously mentioned in this review, B-scan ultrasonography—which is known to be affected by the "blooming effect" that is connected to the lack of a uniform gain setting—was used in all of the included articles [25–54] with the exception of the papers by Dees et al. [30] and Stefanović et al. [42]. Indeed, these papers appear to be the most methodically correct studies because A-scan analysis and the 30-degree test were performed to avoid bias, as previously suggested by Ossoinig [15], even though they are almost 30-year-old and 14-year-old studies, respectively, and they did not analyze a large number of patients.

Considering the "blooming effect", bias may also have an impact on the calipers' position when performing ONSD measurements, changing the objectivity and dependability of the results. Furthermore, the B-scan examination was carried out with the patient's eyelids closed in all of these publications [25–54], which might have resulted in mistakes in the evaluation and measurement of ONSD since the patient's gaze direction was not clearly visible.

For all the above-mentioned B-scan ultrasonography drawbacks, it is recommended to combine the B-scan technique with the standardized A-scan technique [11–23] to provide data that are as exact, unbiased, consistent, and accurate as feasible. In this way, it is possible to undertake a thorough ultrasound examination with far less bias and no loss of important clinical information, leading to more accurate clinical findings.

Currently, regardless of the potential usefulness of ocular ultrasound in the evaluation of the optic nerve, its use is still limited in the management of optic neuropathies, considering the conflicting scientific results and that they can also be identified clinically. However, the role of ocular ultrasound can be decisive in distinguishing a thickening of the optic nerve linked to an inflammatory process or to an increase in subarachnoid fluid, thanks to the use of the "30-degree test" previously described [15].

The main limitations of this review are its non-systematic and basically narrative nature and having used only two scientific databases (PubMed and Scopus) for its drafting.

9. Conclusions

In conclusion, in order to compare clinical findings not just with B-scan ultrasonography but also with other noninvasive procedures that could be helpful in reaching the correct diagnosis in cases of optic neuropathies, future clinical trials and research carried out on this pathological condition should also utilize the standardized A-scan technique. Furthermore, rather than relying on only ultrasound ONSD, it is crucial to highlight that the assessment of optic neuropathies should be carried out in tandem with accessible data from clinical examinations and other diagnostic techniques, correlating them with each other.

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References

1. Biousse, V.; Newman, N.J. Diagnosis and clinical features of common optic neuropathies. *Lancet Neurol.* **2016**, *15*, 1355–1367. [CrossRef] [PubMed]
2. You, Y.; Gupta, V.K.; Li, J.C.; Klistorner, A.; Graham, S.L. Optic neuropathies: Characteristic features and mechanisms of retinal ganglion cell loss. *Rev. Neurosci.* **2013**, *24*, 301–321. [CrossRef] [PubMed]
3. Sandbach, J.M.; Newman, N.J. Retinal masqueraders of optic nerve disease. *Ophthalmol. Clin. N. Am.* **2001**, *14*, 41–59.
4. Weerasinghe, D.; Lueck, C. Mimics and chameleons of optic neuritis. *Pract. Neurol.* **2016**, *16*, 96–110. [CrossRef]
5. Shapey, J.; Sabin, H.I.; Danesh-Meyer, H.V.; Kaye, A.H. Diagnosis and management of optic nerve sheath meningiomas. *J. Clin. Neurosci.* **2013**, *20*, 1045–1056. [CrossRef]
6. Gaier, E.D.; Boudreault, K.; Rizzo, J.F., 3rd; Falardeau, J.; Cestari, D.M. Atypical Optic Neuritis. *Curr. Neurol. Neurosci. Rep.* **2015**, *15*, 76. [CrossRef]
7. Rizzo, J.F., 3rd; Lessell, S. Optic neuritis and ischemic optic neuropathy. Overlapping clinical profiles. *Arch. Ophthalmol.* **1991**, *109*, 1668–1672. [CrossRef]
8. Lochner, P.; Leone, M.A.; Coppo, L.; Nardone, R.; Zedde, M.L.; Cantello, R.; Brigo, F. B-mode transorbital ultrasononography for the diagnosis of acute optic neuritis. A systematic review. *Clin. Neurophysiol.* **2016**, *127*, 803–809. [CrossRef] [PubMed]
9. Rosa, N.; De Bernardo, M.; Di Stasi, M.; Cione, F.; Capaldo, I. A-Scan Ultrasonographic Evaluation of Patients with Idiopathic Intracranial Hypertension: Comparison of Optic Nerves. *J. Clin. Med.* **2022**, *11*, 6153. [CrossRef]
10. Rosa, N.; De Bernardo, M.; Abbinante, G.; Vecchio, G.; Cione, F.; Capasso, L. Optic Nerve Drusen Evaluation: A Comparison between Ultrasound and OCT. *J. Clin. Med.* **2022**, *11*, 3715. [CrossRef]
11. De Bernardo, M.; Vitiello, L.; Rosa, N. A-scan ultrasonography and optic nerve sheath diameter assessment during acute elevations in intra-abdominal pressure. *Surgery* **2020**, *167*, 1023–1024. [CrossRef] [PubMed]
12. De Bernardo, M.; Vitiello, L.; Rosa, N. Optic nerve sheath diameter ultrasonography in differentiation of ischemic and hemorrhagic strokes. *Am. J. Emerg. Med.* **2019**, *37*, 1384–1385. [CrossRef] [PubMed]
13. De Bernardo, M.; Vitiello, L.; Capone, M.; Rosa, N. A-scan ultrasonography and optic nerve sheath diameter evaluation in children with acute liver failure. *Liver Int.* **2020**, *40*, 1504. [CrossRef]
14. De Bernardo, M.; Vitiello, L.; Rosa, N. Ocular ultrasonography to detect intracranial pressure in aneurysmal subarachnoid hemorrhage. *Ann. Clin. Transl. Neurol.* **2020**, *7*, 1459–1460. [CrossRef]
15. Ossoinig, K.C. Standardized echography of the optic nerve. In *Documenta Ophthalmologica Proceedings Series*; Ophthalmic Echography 13; Till, P., Ed.; Springer: Dordrecht, The Netherlands, 1990; Volume 55, pp. 3–99.
16. De Bernardo, M.; Vitiello, L.; De Pascale, I.; Capasso, L.; Cornetta, P.; Rosa, N. Optic Nerve Ultrasound Evaluation in Idiopathic Intracranial Hypertension. *Front. Med.* **2022**, *9*, 845554. [CrossRef] [PubMed]
17. De Bernardo, M.; Vitiello, L.; Rosa, N. Ultrasound optic nerve sheath diameter measurement in optic neuritis. *Am. J. Emerg. Med.* **2019**, *37*, 1595–1596. [CrossRef]
18. De Bernardo, M.; Vitiello, L.; Rosa, N. Ultrasound optic nerve sheath diameter evaluation in patients undergoing robot-assisted laparoscopic pelvic surgery. *J. Robot. Surg.* **2019**, *13*, 709–710. [CrossRef]

19. Abbinante, G.; Vitiello, L.; Coppola, A.; Salerno, G.; Gagliardi, V.; Pellegrino, A. Optic Nerve Ultrasound Evaluation in Children: A Review. *Diagnostics* **2023**, *13*, 535. [CrossRef]
20. Vitiello, L.; De Bernardo, M.; Capasso, L.; Cornetta, P.; Rosa, N. Optic Nerve Ultrasound Evaluation in Animals and Normal Subjects. *Front. Med.* **2022**, *8*, 797018. [CrossRef]
21. De Bernardo, M.; Vitiello, L.; Rosa, N. Optic nerve ultrasound measurement in multiple sclerosis. *Acta Neurol. Scand.* **2019**, *139*, 399–400. [CrossRef]
22. De Bernardo, M.; Vitiello, L.; De Luca, M.; La Marca, A.; Rosa, N. Optic Nerve Changes Detected with Ocular Ultrasonography during Different Surgical Procedures: A Narrative Review. *J. Clin. Med.* **2022**, *11*, 5467. [CrossRef] [PubMed]
23. Vitiello, L.; Salerno, G.; De Bernardo, M.; D'Aniello, O.; Capasso, L.; Marotta, G.; Rosa, N. Ultrasound Detection of Intracranial Hypertension in Brain Injuries. *Front. Med.* **2022**, *9*, 870808. [CrossRef]
24. Kerlen, C.H. B-scan ultrasonography in optic nerve lesions. *Doc. Ophthalmol.* **1982**, *52*, 317–325. [CrossRef]
25. Abegão Pinto, L.; Vandewalle, E.; Pronk, A.; Stalmans, I. Intraocular pressure correlates with optic nerve sheath diameter in patients with normal tension glaucoma. *Graefes Arch. Clin. Exp. Ophthalmol.* **2012**, *250*, 1075–1080. [CrossRef] [PubMed]
26. Willekens, K.; Abegão Pinto, L.; Vandewalle, E.; Marques-Neves, C.; Stalmans, I. Higher optic nerve sheath diameters are associated with lower ocular blood flow velocities in glaucoma patients. *Graefes Arch. Clin. Exp. Ophthalmol.* **2014**, *252*, 477–483. [CrossRef]
27. Liu, H.; Yang, D.; Ma, T.; Shi, W.; Zhu, Q.; Kang, J.; Wang, N. Measurement and Associations of the Optic Nerve Subarachnoid Space in Normal Tension and Primary Open-Angle Glaucoma. *Am. J. Ophthalmol.* **2018**, *186*, 128–137. [CrossRef] [PubMed]
28. Omatiga, A.G.; Onakpoya, O.H.; Idowu, B.M.; Asaleye, C.M.; Adegbehingbe, B.O.; Aderibigbe, A.S. B-mode sonographic evaluation of optic nerve sheath diameter and lens thickness in Nigerian adults with glaucoma. *Afr. Health Sci.* **2018**, *18*, 343–351. [CrossRef] [PubMed]
29. Coleman, D.J.; Carroll, F.D. Evaluation of optic neuropathy with B-scan ultrasonography. *Am. J. Ophthalmol.* **1972**, *74*, 915–920. [CrossRef]
30. Dees, C.; Buimer, R.; Dick, A.D.; Atta, H.R. Ultrasonographic investigation of optic neuritis. *Eye* **1995**, *9 Pt 4*, 488–494. [CrossRef]
31. Elvin, A.; Andersson, T.; Söderström, M. Optic neuritis. Doppler ultrasonography compared with MR and correlated with visual evoked potential assessments. *Acta Radiol.* **1998**, *39*, 243–248.
32. Karaali, K.; Senol, U.; Aydin, H.; Cevikol, C.; Apaydin, A.; Lüleci, E. Optic neuritis: Evaluation with orbital Doppler sonography. *Radiology* **2003**, *226*, 355–358. [CrossRef]
33. Karami, M.; Janghorbani, M.; Dehghani, A. Orbital Doppler evaluation of blood flow velocities in optic neuritis. *Korean J. Ophthalmol.* **2012**, *26*, 116–122. [CrossRef]
34. Lochner, P.; Cantello, R.; Fassbender, K.; Lesmeister, M.; Nardone, R.; Siniscalchi, A.; Clemente, N.; Naldi, A.; Coppo, L.; Brigo, F.; et al. Longitudinal Assessment of Transorbital Sonography, Visual Acuity, and Biomarkers for Inflammation and Axonal Injury in Optic Neuritis. *Dis. Markers* **2017**, *2017*, 5434310. [CrossRef]
35. Lochner, P.; Cantello, R.; Brigo, F.; Coppo, L.; Nardone, R.; Tezzon, F.; Raymkulova, O.; Strigaro, G.; Comi, C.; Leone, M.A. Transorbital sonography in acute optic neuritis: A case-control study. *AJNR Am. J. Neuroradiol.* **2014**, *35*, 2371–2375. [CrossRef] [PubMed]
36. Lochner, P.; Leone, M.A.; Fassbender, K.; Cantello, R.; Coppo, L.; Nardone, R.; Zorzi, G.; Lesmeister, M.; Comi, C.; Brigo, F. Transorbital Sonography and Visual Outcome for the Diagnosis and Monitoring of Optic Neuritis. *J. Neuroimaging* **2017**, *27*, 92–96. [CrossRef]
37. Wayman, D.; Carmody, K.A. Optic neuritis diagnosed by bedside emergency physician-performed ultrasound: A case report. *J. Emerg. Med.* **2014**, *47*, 301–305. [CrossRef] [PubMed]
38. Badron, J.; Ong, G.Y. Bedside Transorbital Ultrasound in the Clinical Evaluation of Pediatric Optic Neuritis in the Emergency Department. *J. Emerg. Med.* **2019**, *56*, 417–420. [CrossRef] [PubMed]
39. Yee, N.P.; Kashani, S.; Mailhot, T.; Omer, T. More than meets the eye: Point-of-care ultrasound diagnosis of acute optic neuritis in the emergency department. *Am. J. Emerg. Med.* **2019**, *37*, 177.e1–177.e4. [CrossRef]
40. Rovira, À.; Vidal-Jordana, A.; Auger, C.; Sastre-Garriga, J. Optic Nerve Imaging in Multiple Sclerosis and Related Disorders. *Neuroimaging Clin. N. Am.* **2024**, *34*, 399–420. [CrossRef]
41. Titlić, M.; Erceg, I.; Kovacević, T.; Gabrić, N.; Karaman, K.; Zuljan, I.; Orsolić, K.; Kalajzić, J. The correlation of changes of the optic nerve diameter in the acute retrobulbar neuritis with the brain changes in multiple sclerosis. *Coll. Antropol.* **2005**, *29*, 633–636.
42. Stefanović, I.B.; Jovanović, M.; Krnjaja, B.D.; Veselinović, D.; Jovanović, P. Influence of retrobulbar neuritis and papillitis on echographically measured optic nerve diameter. *Vojnosanit. Pregl.* **2010**, *67*, 32–35. [CrossRef]
43. Raeesmohammadi, L.; Esmaeili, S.; Abbasi, M.H.; Mehrpour, M.; Mirzaasgari, Z.; Baradaran, H.R.; Deilami, P.; Motamed, M.R. Transbulbar B-mode sonography in multiple sclerosis without optic neuritis; clinical relevance. *Brain Res.* **2020**, *1734*, 146723. [CrossRef] [PubMed]
44. De Masi, R.; Orlando, S.; Conte, A.; Pasca, S.; Scarpello, R.; Spagnolo, P.; Muscella, A.; De Donno, A. Transbulbar B-Mode Sonography in Multiple Sclerosis: Clinical and Biological Relevance. *Ultrasound Med. Biol.* **2016**, *42*, 3037–3042. [CrossRef] [PubMed]

45. Schroeder, C.; Katsanos, A.H.; Ayzenberg, I.; Schwake, C.; Gahlen, A.; Tsivgoulis, G.; Voumvourakis, K.; Gold, R.; Krogias, C. Atrophy of optic nerve detected by transorbital sonography in patients with demyelinating diseases of the central nervous system. *Eur. J. Neurol.* **2020**, *27*, 626–632. [CrossRef]
46. Elkholy, S.H.; El-Jaafary, S.I.; Kotb, M.S.; El Gohary, A.M.; Elbhy, B.A. Trans-orbital sonography versus visual evoked potentials in acute demyelinating optic neuritis. *Mult. Scler. Relat. Disord.* **2020**, *40*, 101934. [CrossRef]
47. Saigh, M.P.; Plauché, H.M.; Butts, C.; Karam, A.K.; Suau, S.J.; Moreno-Walton, L. Acute Optic Neuritis Diagnosed by Bedside Ultrasound in an Emergency Department. *J. Emerg. Med.* **2019**, *57*, 207–211. [CrossRef] [PubMed]
48. Kwon, Y.J.; Kim, Y.H.; Baek, S.H.; Son, M.H.; Lee, J.H.; Kim, B.J. Transorbital ultrasonography in acute optic neuritis: Can it be a supportive diagnostic tool? *Mult. Scler. Relat. Disord.* **2019**, *31*, 54–58. [CrossRef]
49. Koraysha, N.A.; Kishk, N.; Hassan, A.; Samy El Gendy, N.M.; Shehata, H.S.; Al-Azayem, S.A.; Kamal, Y.S. Evaluating optic nerve diameter as a possible biomarker for disability in patients with multiple sclerosis. *Neuropsychiatr. Dis. Treat.* **2019**, *15*, 2571–2578. [CrossRef]
50. Krogias, C.; Ayzenberg, I.; Schroeder, C.; Grüter, T.; Gold, R.; Yoon, M.S. Transorbital sonography in CIDP patients: No evidence for optic nerve hypertrophy. *J. Neurol. Sci.* **2016**, *362*, 206–208. [CrossRef]
51. Candeliere Merlicco, A.; Gabaldón Torres, L.; Villaverde González, R.; Fernández Romero, I.; Aparicio Castro, E.; Lastres Arias, M.C. Transorbital ultrasonography for measuring optic nerve atrophy in multiple sclerosis. *Acta Neurol. Scand.* **2018**, *138*, 388–393. [CrossRef]
52. Gerling, J.; Janknecht, P.; Hansen, L.L.; Kommerell, G. Diameter of the optic nerve in idiopathic optic neuritis and in anterior ischemic optic neuropathy. *Int. Ophthalmol.* **1997**, *21*, 131–135. [CrossRef] [PubMed]
53. Dehghani, A.; Giti, M.; Akhlaghi, M.R.; Karami, M.; Salehi, F. Ultrasonography in distinguishing optic neuritis from nonarteritic anterior ischemic optic neuropathy. *Adv. Biomed. Res.* **2012**, *1*, 3. [CrossRef] [PubMed]
54. Ji, X.; Xiao, W.; Ye, H.; Chen, R.; Wu, J.; Mao, Y.; Yang, H. Ultrasonographic measurement of the optic nerve sheath diameter in dysthyroid optic neuropathy. *Eye* **2021**, *35*, 568–574. [CrossRef] [PubMed]

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Subclinical Ocular Motility Dysfunction and Extraocular Muscle Changes in Inactive Graves' Orbitopathy

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Abstract: This study aimed to investigate the presence of structural and functional changes in extraocular muscles (EMs) among patients with inactive Graves' orbitopathy (GO) classified according to the Clinical Activity Score (CAS). Sixty-seven patients with Graves' disease (GD) and inactive GO were included. The data collected included clinical parameters, thyroid function, autoantibody levels, EOM morphology via orbital ultrasound (US), and ocular motility. Patients were stratified into Red Filter Test (RFT)-positive or RFT-negative groups based on the presence or absence of latent diplopia during the RFT examination. Thirty-three patients (49.25%) exhibited latent diplopia on the RFT, despite not reporting double vision during standard ocular motility tests. Significant differences were observed between the two groups in terms of age, disease duration, intraocular pressure (IOP) elevation in up-gaze, and medial rectus muscle thickness ($p < 0.05$). No significant differences were found in thyroid status, TRAb and ATA levels, CASs, exophthalmos, or lateral rectus thickness between the two groups. This study revealed that in inactive GO, subclinical EM dysfunction and morphological changes may be present, which might not be apparent through routine ocular examinations. The RFT is effective in detecting latent diplopia, highlighting its utility in identifying subtle ocular motility issues and subclinical muscle involvement. Comprehensive evaluations combining functional tests like the RFT and imaging are essential for early detection of GO-related abnormalities, enabling tailored and prompt management and improving patient outcomes.

Keywords: thyroid eye disease; Graves' orbitopathy; Red Filter Test; CAS

1. Introduction

Graves' Disease (GD) is an autoimmune disorder, known as the leading cause of hyperthyroidism, responsible for 50–80% of cases globally [1–3]. The disease is typically triggered by thyrotropin receptor autoantibodies (TRAb), which bind to and activate the thyroid-stimulating hormone receptor (TSHR), causing an overproduction of thyroid hormones [4].

Graves' orbitopathy (GO) is the most frequent extra-thyroidal manifestation of GD, affecting about 50% of those with Graves' hyperthyroidism. It is noteworthy that individuals with Hashimoto's thyroiditis or even those without evident thyroid disease can also develop GO [5,6].

As an immune-mediated disease, GO is triggered by auto-reactive T lymphocytes recognizing one or more antigens shared by the thyroid and orbital tissues, primarily the TSHR [7]. This inflammatory process causes the expansion of orbital fibro-adipose tissue, the infiltration and enlargement of extraocular muscles (EMs), leading to clinical manifestations such as exophthalmos, diplopia, ocular soft tissue changes, and possible optic nerve compression [8,9].

The Clinical Activity Score (CAS), proposed by Mourits and colleagues, is based on a set of criteria, with each criterion being scored as present or absent, and aims to distinguish patients with active GO from those with inactive disease [10].

However, it is well known that GD patients with inactive GO may present morphologic alterations of EMs and/or fibro-adipose tissue changes detectable with different imagine techniques [11]. Moreover, the dysfunction of EMs can cause eye movement-related impairment, significantly altering the quality of patients' life and presenting as a treatment challenge [12].

This study aimed to investigate the presence of alteration in EMs' function, evaluated by means of the Red Filter test (RFT) among patients with inactive Graves' orbitopathy (GO) without frank diplopia.

2. Materials and Methods

This was a retrospective study conducted at the University Hospital of Cagliari, Italy. The study received approval by the Institutional Review Board (IRB) of the University of Cagliari (PG/2015/3455; 26 February 2015). All subjects provided written informed consent and the study was performed in accordance with the Declaration of Helsinki.

2.1. Patients' Selection and Data Collection

A total of 67 consecutive patients with GD and inactive GO according to CAS were examined. Patients were enrolled consecutively between January 2011 and December 2014 at a single center (University Hospital of Cagliari, Cagliari, Italy). The following data were collected from each patient's medical file: age, sex, disease duration, CAS class, free thyroxine (FT4), free triiodothy-ronine (FT3) and thyroid-stimulating hormone (TSH), thyrotropin receptor antibody (TRAb) [TRAb levels were considered as a low titer (1–2 IU/L), moderate titer (2–10 IU/L) and high titer (>10 IU/L)], Anti-Thyroid Antibody (ATA) levels [ATA levels were considered positive if anti-thyroglobulin (TG) antibodies were equal to or higher than 4.5 IU/mL, or if anti-thyroid peroxidase (TPO) antibodies were equal to or higher than 60 IU/mL], intraocular pressure (IOP) measurement in primary and up-gaze for both eyes, best-corrected visual acuity (BCVA), slit lamp examination, eye motility, RFT and exophthalmometry. Patients were categorized into 2 groups based on positive (RFT-positive) or negative diplopia (RFT-negative) on RFT.

2.2. Ophthalmic Assessment

Ocular motility tests were performed by the same experienced ophthalmologist (A.C.) to evaluate latent or manifest deficits. Manifest diplopia was assessed by asking the patients to follow a small tool moved from the center of the visual field towards its periphery in the four main directions. The presence of latent diplopia was investigated using the RFT: a red glass was placed in front of the patient's right eye, and the patient was asked to focus on a single white light source directly in front of them, maintaining a primary gaze. The patient was then instructed to look at the light source from various positions of gaze, including the primary position and the eight cardinal positions. If there was a muscle dysfunction, the patient reported diplopia (perception of two lights: white and red). The direction of gaze that resulted in diplopia or showed the greatest separation of the images could suggest which structures were involved. IOP was measured first in primary gaze and then in up-gaze. The measurement of eye proptosis was assessed with Hertel's exophtalmometer. The normal range of values was considered 12–21 mm. Values above the upper limit or the difference between the two eyes $\geq$ 3 mm were considered positive.

2.3. Imaging Evaluation

Orbital US was performed by the same experienced echographer (A.C.) using a B-mode MYLAB 70XV Echo-scan (ESAOTE, Genova, Italy) with a 10 MHz transducer. The medial and lateral rectus muscles were examined in both eyes, while the vertical recti were measured only occasionally. During the examination, the patients were asked to look straight ahead, maintaining a primary gaze position. The probe was placed on the closed eyelid, on the opposite side of the muscle that was examined. Suitable sections were frozen on the screen when the trans-bulbar muscle stripe appeared as distinctly as possible. The thickest section of the muscle was then measured at the point of greatest enlargement, perpendicular to the muscle axis, using calipers. Muscles were considered normal if the thickness was < 4 mm, thickened if the thickness was between 4 and 6 mm, and very thickened if the thickness was > 6 mm.

2.4. Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Science software (IMB SPSS Statistics, version 25 for Windows). Means ± standard deviations (SDs)/median for continuous variables were estimated or percent distributions were presented. The distribution of variables was assessed with Shapiro–Wilk tests. Visual acuity was converted in logMAR for statistical analysis. Fisher's test and Chi-squared test were used to compare categorical variables. Parametric (*t*-test) and non-parametric (Mann–Whitney U test) tests were used to compare normally and non-normally distributed variables, respectively, between groups. A p value of <0.05 was considered statistically significant.

3. Results

3.1. Demographic, Laboratories and Clinical Characteristics of Study Population

All patients were Caucasians. A total of 134 eyes of 67 patients were included in this study. The medical records of 46 females and 21 males with a mean age 46.0 ± 12.1 (range 18–72 years) were evaluated. Thirty-six (53.73%) patients were hyperthyroid, twenty-eight (41.79%) were euthyroid, and three (4.47%) were hypothyroid. The mean duration of thyroid disease was 29.00 ± 41.21 months. TSH mean value was 1.57 mU/L (median 0.50 mU/L), FT4 mean value was 2.45 ng/dL (median 1.40 ng/dL), and FT3 mean value was 4.47 pg/mL (median 3.36 pg/mL). TRAb values were positive in 51 (76.11%) patients, with a low titer in 22 subjects (32.83%), moderate titer in 16 (23.88%) and high titer in 13 (19.40%). Sixteen (23.88%) patients were negative for TRAb. ATAs were positive in 48 (71.64%) and negative in 19 (28.36%) patients. All patients presented CAS ≤ 2. Thirty-six (53.73%) patients were CAS 0, twenty-six (38.80%) were CAS 1 and five (7.46%) were CAS 2. Eight (11.94%) patients presented mild exophthalmos (1–2 mm proptosis above the upper limit or 3–4 mm of difference compared to the fellow eye). No patients complained of diplopia upon evaluation of ocular motility. BCVA was logMAR 0.02 ± 0.09. Mean IOP in primary gaze was 14.46 ± 2.76 mmHg. Mean IOP in up-gaze was 16.64 ± 4.07. In the US exam, considering both orbits, medial rectus resulted normal in 69 (51.49%), thickened in 57 (42.54%) and very thickened in 8 eyes (5.97%). Lateral rectus was normal in 109 (81.34%), thickened in 22 (16.42%) and very thickened in 3 eyes (2.24%). During the ocular motility examination, no patients reported manifest diplopia. Conversely, in the RFT, 33 (49.25%) patients exhibited latent diplopia in the vertical gaze position.

Demographic, laboratory and clinical characteristics are displayed in Table 1.

Table 1. Demographic, laboratory and clinical data of patients.

	All Patients (n = 67) ***n (%)***
Gender	
Male	21 (31.3%)
Female	46 (68.7%)
Mean age (SD)	46 (12.1)
Thyroid status	
Hyperthyroid	36 (53.7%)
Euthyroid	28 (41.8%)
Hypothyroid	3 (4.4%)
Mean duration of disease (months)	29.0 (41.21)
Mean thyroid hormones (SD)	
TSH (mU/L)	1.57 (3.02)
FT4 (ng/dL)	2.45 (3.90)
FT3 (pg/mL)	4.47 (4.47)
TRAb title	
Negative	16 (22.8%)
Low(1–2 IU/L)	22 (32.8%)
Moderate (2–10 IU/L)	16 (22.8%)
High (>10 IU/L)	13 (19.4%)
ATA levels	
Positive (Anti-TG ≥ 4.5 IU/mLorAnti-TPO ≥ 60 IU/mL)	48 (71.6%)
Negative (Anti-TG < 4.5 IU/mLorAnti-TPO < 60 IU/mL)	19 (28.4%)
CAS	
CAS 0	36 (53.7%)
CAS 1	26 (38.8%)
CAS 2	5 (7.4%)
Exophthalmos (>21 mm or difference between the two eyes ≥ 3 mm)	8 (11.9%)
BCVA (logMAR) (SD)	0.02 (0.09)
Mean IOP (mmHg) (SD)	
Primary gaze	14.46 (2.76)
Up-gaze	16.64 (4.07)
US Medial Rectus	
Normal (<4 mm)	69 (51.5%)
Thickened (4–6 mm)	57 (42.5%)
Very Thickened (>6 mm)	8(6.0%)
US Lateral Rectus	
Normal (<4 mm)	109 (81.3%)
Thickened (4–6 mm)	22 (16.4%)
Very Thickened (>6 mm)	3 (2.2%)

TSH = thyroid-stimulating hormone; FT4 = free thyroxine; FT3 = free triiodothy-ronine; TRAb = thyrotropin receptor antibody; Anti-TG = Anti-thyroglobulin; Anti-TPO = Anti-thyroid peroxidase; CAS = Clinical Activity Score; BCVA = Best-corrected visual acuity; IOP = Intraocular pressure; US = Ultrasound.

3.2. Analysis of RFT-Positive vs. RFT-Negative Patients

Study subjects were divided into two groups according to positive (RFT-positive, n = 33) or negative diplopia on RFT (RFT-negative, n = 34). The characteristics of two groups are displayed in Table 2.

Table 2. Demographic, laboratory and clinical characteristics of two groups.

	RFT-Positive (n = 33) n (%)	***RFT-Negative (n = 34) n (%)***	***p-Value***
Gender			1 *
Male	10 (30.3%)	11 (32.4%)	
Female	23 (69.7%)	23 (67.6%)	
Mean age (SD)	49.1 (12.3)	43.0 (11.3)	0.03 †
Thyroid status			0.75 x
Hyperthyroid	17 (51.5%)	19 (55.9%)	
Euthyroid	15 (45.5%)	13 (38.2%)	
Hypothyroid	1 (3.0%)	2 (5.9%)	
Mean duration of disease (months) (SD)	34.1 (37.6)	24.1 (44.5)	0.04 ‡
Mean thyroid hormones (SD)			
TSH (mU/L)	1.03 (1.27)	2.10 (4.08)	0.71 ‡
FT4 (ng/dL)	2.38 (3.17)	2.52 (4.54)	0.23 ‡
FT3 (pg/mL)	5.13 (5.85)	3.83 (2.45)	0.23 ‡
TRAb title			0.55 x
Negative	6 (18.2%)	10 (29.4%)	
Low (1–2 IU/L)	10 (30.3%)	12 (35.3%)	
Moderate (2–10 IU/L)	9 (27.3%)	7 (20.6%)	
High (>10 IU/L)	8 (24.2%)	5 (14.7%)	
ATA levels			1 *
Positive (Anti-TG ≥ 4.5 IU/mLorAnti-TPO ≥ 60 IU/mL)	24 (72.7%)	24 (70.6%)	
Negative (Anti-TG < 4.5 IU/mLorAnti-TPO < 60 IU/mL)	9 (27.3%)	10 (29.4%)	
CAS			0.30 x
CAS 0	18 (54.6%)	18 (53.0%)	
CAS 1	11 (33.3%)	15 (44.1%)	
CAS 2	4 (12.1%)	1 (2.9%)	
Exophthalmos (>21 mm or difference between the two eyes ≥ 3 mm)	3 (9.1%)	5 (14.7%)	0.71 *
BCVA (logMAR)(SD)	0.01 (0.01)	0.03 (0.01)	0.27 ‡
Mean IOP (mmHg) (SD)			
Primary gaze	14.20 (2.80)	14.71 (2.72)	0.46 ‡
Up-gaze	18.15 (4.56)	15.18 (2.87)	0.00005 ‡
US Medial Rectus			0.003 x
Normal (<4 mm)	27 (40.9%)	42 (61.8%)	
Thickened (4–6 mm)	31 (47.0%)	26 (38.2%)	
Very Thickened (>6 mm)	8 (12.1%)	0 (0.0%)	
US Lateral Rectus			0.10 x
Normal (<4 mm)	50 (75.8%)	59 (86.8%)	
Thickened (4–6 mm)	13 (19.7%)	9 (13.2%)	
Very Thickened (>6 mm)	3 (4.5%)	0 (0.0%)	

* = Fisher's exact test; † = *t*-test; ‡ = Mann–Whitney U test; x = Chi-squared test. TSH = thyroid-stimulating hormone; FT4 = free thyroxine; FT3 = free triiodothy-ronine; TRAb = thyrotropin receptor antibody; Anti-TG = Anti-thyroglobulin; Anti-TPO = Anti-thyroid peroxidase; CAS = Clinical Activity Score; BCVA = Best-corrected visual acuity; IOP = Intraocular pressure; US = Ultrasound.

The gender distribution was similar across both groups ($p = 1$). The RFT-positive group had a significantly higher mean age (49.1 ± 12.3 years vs. 43.0 ± 11.3 years, $p = 0.03$) and a longer mean disease duration (34.1 ± 37.6 months vs. 24.1 ± 44.5 months, $p = 0.04$). Thyroid status, thyroid hormone levels (TSH, FT4, FT3), TRAb levels, ATA, CASs, exophthalmos presence and BCVA showed no significant differences between the groups. The mean IOP exhibited a significant increase from 14.20 ± 2.80 mmHg in primary gaze to 18.15 ± 4.56 mmHg in up-gaze ($p < 0.001$, Mann–Whitney U test), corresponding to a 27.8% elevation in the RFT-positive group. Concerning the RFT-negative group, the mean IOP exhibited an increase from

14.71 ± 2.72 mmHg in primary gaze to 15.18 ± 2.87 mmHg in up-gaze ($p = 0.35$, Mann–Whitney U test), corresponding to a 3.2% elevation in the RFT-negative group. In up-gaze, the mean IOP was significantly higher in the RFT-positive group compared to the RFT-negative group ($p = 0.00005$). Ultrasound measurements revealed that the medial rectus muscle was more frequently thickened in the RFT-positive group (59.1% vs. 38.2%, $p = 0.003$), while the lateral rectus muscle thickness did not differ significantly between the groups (24.2% vs. 13.2, $p = 0.10$).

4. Discussion

External eye muscles play a critical role in the pathophysiology of GO, being involved in both active and inactive phases of the disease. In these conditions, the muscles may become edematous and infiltrated or undergo fibrosis, which can alter eye motility [12,13].

Double vision or diplopia is a common and debilitating symptom of GO [14]. The European Group on Grave's Orbitopathy (EU-GOGO) reported that 49% of patients with GO presented some degree of eye movement dysfunction [15]. Additionally, patients with diplopia pose a major burden for public health systems due to potential occupational impairments, especially when double vision occurs in the primary and reading position [16].

Diplopia may be latent or manifest. The former occurs when the eyes tend to deviate from their normal position, but this misalignment is typically kept in check by the brain's fusion mechanism. In latent diplopia, double vision does not usually occur because the brain compensates and corrects the misalignment. However, under certain conditions, the brain's ability to maintain proper alignment can weaken, potentially leading to eye strain or double vision. Conversely, manifest diplopia is a condition where there is a constant and noticeable misalignment of the eyes, leading to persistent double vision which the brain cannot correct [17].

Several classifications have been developed and used to evaluate GO. VISA and EUGOGO both subjectively assess the presence of diplopia, noting whether it is vertical or horizontal, constant or intermittent, and if it worsens in the morning [18]. None of these scores can detect latent diplopia or measure the level of involvement of all EMs throughout the eye disease. This gap in the ocular evaluations highlights the need for some methods to be implemented in current practice to comprehensively assess diplopia. Accordingly, while the Hess Lancaster test is considered the gold standard for evaluating diplopia, it requires specialized equipment and trained technicians. In contrast, the RFT is a straightforward, cost-effective, and reliable method that can be easily performed in a clinical setting without specialized instruments [19]. Therefore, RFT can offer a valid alternative to rapidly and non-invasively evaluating diplopia in these patients.

In the studied populations, 33 (49.25%) patients with inactive GO exhibited positive diplopia on RFT in vertical gaze, which they had previously not complained of during the ocular motility examination. This could be due to the ability of RFT to evaluate the total deviation (both manifest and latent) by disrupting fusional mechanisms through stark color differences between the eyes [20]. This finding highlights that even in the absence of active disease, subclinical changes in EM function can still be present. Therefore, while CAS is an effective score for categorizing disease activity, it may not fully capture subtle changes in muscle morphology or function.

In terms of structural alterations, a significant difference in IOP between primary and up-gaze positions is well-established in patients with GO as an indicator of inferior rectus muscle thickening. Specifically, in the up-gaze position, the pulling of inferior rectus muscle in patients with GO causes the superior rectus muscle to use greater force to elevate the eyeball. This additional force acts on the eyeball wall and the compression of the superior and inferior rectus muscle significantly elevates the IOP [21]. Despite the lack of a thickness measurement of the vertical recti in our cohort due to different limitations of US in evaluating superior and inferior rectus [22], the significant IOP increment in the RF-positive group may reflect increased resistance from a stiffened inferior rectus muscle.

Ultrasounds represent a cheap, rapid and effective tool for assessing eye muscle involvement in patients with GO, especially compared with more invasive exams like computed tomography (CT) and magnetic resonance imaging (MRI) [23].

B-scan images showed that the horizontal recti had different thickening rates between the two groups, with more thickening of the medial rectus in the RFT-positive group. This finding is consistent with the literature, which indicates that the medial rectus is primarily involved in GO, after the inferior rectus [24]. No significant difference was observed in the lateral rectus thickness between the two groups, likely because the latter is the least affected muscle in GO, followed by the oblique muscles. [25]. Despite these results, the RFT was positive in vertical gaze, likely because the maximum vertical disparity that can be fused is only about 3–5 Δ, significantly less than the horizontal fusion amplitude of 15–20 Δ [26].

The factors of disease duration and age of patients exhibited a significant difference between the two groups. These could lead to an increased cumulative exposure to autoimmune inflammation and subsequently more significant tissue damage and fibrosis over time, which could be reflected in imaging and functional test alterations. Additionally, age-related changes in the ocular muscles and overall tissue elasticity reduction could contribute to the observed differences between the groups [27].

Patients with GO may have hyperthyroidism, hypothyroidism, or euthyroidism [5]. The distribution of thyroid status did not significantly differ between the RFT-positive and RFT-negative groups, suggesting that latent diplopia is not directly influenced by the current thyroid status. Similarly, sex and patients with some degree of exophthalmos did also not differ between the two groups.

The role of TRAb and ATA in the pathogenesis of GD and GO has been extensively investigated [28]. However, the lack of a significant difference between the groups suggests that these antibodies alone do not determine the presence of diplopia, as already shown in previous reports [29,30].

These findings suggest that even in inactive GO, there can be subtle yet clinically relevant changes in EOM function and morphology. The early detection of these changes could significantly influence the progression and prognosis of the disease by facilitating a dedicated management plan to mitigate disease advancement. This plan might involve more rigorous follow-up schedules to monitor the patient closely and implement timely interventions. Preventive measures, such as protecting the eyes from sun and wind exposure and encouraging patients to quit smoking, can help reduce risk factors associated with disease exacerbation [31]. Ensuring optimal thyroid hormone levels by tailoring anti-thyroid therapy to achieve euthyroidism and avoiding hormonal fluctuations is also crucial in stabilizing the condition. Furthermore, considering selenium's potential benefits in slowing disease progression, initiating therapy with 200 mcg/day could be a viable option for such patients [32]. Through these strategies, early intervention may improve disease progression and patient outcomes.

Nonetheless, the study had several limitations. Firstly, the retrospective design of the study inherently limits the generalizability of our findings. Secondly, the research was conducted at a single center with a cohort composed entirely of Caucasian patients, which may restrict the generalizability of the results to other populations or settings. Thirdly, the relatively small sample size of 67 patients could affect the robustness and statistical power of the study [the statistical power of the study, calculating by using G*Power software (version 3.1.9.6) [33], given a total sample size of 67 (with groups of 33 and 34 participants) a significance level of 0.05, and an effect size of 0.5, is approximately 0.65]. Additionally, the reliance on the RFT for assessing latent diplopia may not account for all potential functional deficits, suggesting the need for additional functional assessments in future research.

5. Conclusions

The present study identifies subclinical EM dysfunction and changes in inactive GO patients. Clinicians should consider comprehensive ocular evaluations, including imaging

studies and functional tests like the RFT, which can be integrated into the standard VISA or EUGOGO evaluation to facilitate a better assessment of the extent of EOM involvement in GO. The early detection of latent diplopia and muscular abnormalities, as well as appropriate therapeutic options, might influence the progression and prognosis of more severe manifestations of late fibrotic alterations, thereby improving patient outcomes. Future research should focus on larger, multi-center studies with advanced imaging and functional assessments to gain a deeper understanding of EM dysfunction in inactive GO, and refine treatment approaches.

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References

1. Brent, G.A. Clinical practice. Graves' disease. *N. Engl. J. Med.* **2008**, *358*, 2594–2605. [CrossRef] [PubMed]
2. Cooper, D.S. Hyperthyroidism. *Lancet* **2003**, *362*, 459–468. [CrossRef] [PubMed]
3. Weetman, A.P. Graves' Disease. *N. Engl. J. Med.* **2000**, *343*, 1236–1248. [CrossRef] [PubMed]
4. Eckstein, A.K.; Johnson, K.T.M.; Thanos, M.; Esser, J.; Ludgate, M. Current insights into the pathogenesis of Graves' orbitopathy. *Horm. Metab. Res.* **2009**, *41*, 456–464. [CrossRef] [PubMed]
5. Bartalena, L.; Tanda, M.L. Current concepts regarding Graves' orbitopathy. *J. Intern. Med.* **2022**, *292*, 692–716. [CrossRef] [PubMed] [PubMed Central]
6. Bartalena, L.; Tanda, M.L. Clinical practice. Graves' ophthalmopathy. *N. Engl. J. Med.* **2009**, *360*, 994–1001. [CrossRef] [PubMed]
7. Scarabosio, A.; Surico, P.L.; Singh, R.B.; Tereshenko, V.; Musa, M.; D'Esposito, F.; Russo, A.; Longo, A.; Gagliano, C.; Agosti, E.; et al. Thyroid Eye Disease: Advancements in Orbital and Ocular Pathology Management. *J. Pers. Med.* **2024**, *14*, 776. [CrossRef] [PubMed] [PubMed Central]
8. Bahn, R.S. Graves' Ophthalmopathy. *N. Engl. J. Med.* **2010**, *362*, 726–738. [CrossRef] [PubMed]
9. Smith, T.J. Pathogenesis of Graves' orbitopathy: A 2010 update. *J. Endocrinol. Investig.* **2010**, *33*, 414–421. [CrossRef]
10. Mourits, M.P.; Koornneef, L.; Wiersinga, W.M.; Prummel, M.F.; Berghout, A.; van der Gaag, R. Clinical criteria for the assessment of disease activity in Graves' ophthalmopathy: A novel approach. *Br. J. Ophthalmol.* **1989**, *73*, 639–644. [CrossRef]
11. Lennerstrand, G.; Tian, S.; Isberg, B.; Landau Högbeck, I.; Bolzani, R.; Tallstedt, L.; Schworm, H. Magnetic resonance imaging and ultrasound measurements of extraocular muscles in thyroid-associated ophthalmopathy at different stages of the disease. *Acta Ophthalmol. Scand.* **2007**, *85*, 192–201. [CrossRef] [PubMed]
12. Nagy, E.V.; Toth, J.; Kaldi, I.; Damjanovich, J.; Mezosi, E.; Lenkey, A.; Toth, L.; Szabo, J.; Karanyi, Z.; Leovey, A. Graves' ophthalmopathy: Eye muscle involvement in patients with diplopia. *Eur. J. Endocrinol.* **2000**, *142*, 591–597. [CrossRef] [PubMed]
13. Jankauskiene, J.; Imbrasiene, D. Investigations of ocular changes, extraocular muscle thickness, and eye movements in Graves' ophthalmopathy. *Medicina* **2006**, *42*, 900–903. [PubMed]
14. Johnson, B.T.; Jameyfield, E.; Aakalu, V.K. Optic neuropathy and diplopia from thyroid eye disease: Update on pathophysiology and treatment. *Curr. Opin. Neurol.* **2021**, *34*, 116–121. [CrossRef] [PubMed] [PubMed Central]
15. Prummel, M.F.; Bakker, A.; Wiersinga, W.M.; Baldeschi, L.; Mourits, M.P.; Kendall-Taylor, P.; Perros, P.; Neoh, C.; Dickinson, A.J.; Lazarus, J.H.; et al. Multi-center study on the characteristics and treatment strategies of patients with Graves' orbitopathy: The first European Group on Graves' Orbitopathy experience. *Eur. J. Endocrinol.* **2003**, *148*, 491–495. [CrossRef] [PubMed]
16. Ponto, K.A.; Merkesdal, S.; Hommel, G.; Pitz, S.; Pfeiffer, N.; Kahaly, G.J. Public health relevance of Graves' orbitopathy. *J. Clin. Endocrinol. Metab.* **2013**, *98*, 145–152. [CrossRef] [PubMed]
17. Gräf, M.; Lorenz, B. How to deal with diplopia. *Rev. Neurol.* **2012**, *168*, 720–728. [CrossRef] [PubMed]

18. Barrio-Barrio, J.; Sabater, A.L.; Bonet-Farriol, E.; Velázquez-Villoria, Á.; Galofré, J.C. Graves' Ophthalmopathy: VISA versus EUGOGO Classification, Assessment, and Management. *J. Ophthalmol.* **2015**, *2015*, 249125. [CrossRef] [PubMed] [PubMed Central]
19. Yoo, H.S.; Park, E.; Rhiu, S.; Chang, H.J.; Kim, K.; Yoo, J.; Heo, J.H.; Nam, H.S. A computerized red glass test for quantifying diplopia. *BMC Ophthalmol.* **2017**, *17*, 71. [CrossRef] [PubMed] [PubMed Central]
20. Bitner, D.P.; Adesina, O.O.; Ding, K.; Farris, B.K.; Michael Siatkowski, R. Comparison of Objective and Subjective Techniques of Strabismus Measurement in Adults With Normal Retinal Correspondence. *J. Pediatr. Ophthalmol. Strabismus.* **2017**, *54*, 216–220. [CrossRef] [PubMed]
21. Li, X.; Bai, X.; Liu, Z.; Cheng, M.; Li, J.; Tan, N.; Yuan, H. The Effect of Inferior Rectus Muscle Thickening on Intraocular Pressure in Thyroid-Associated Ophthalmopathy. *J. Ophthalmol.* **2021**, *2021*, 9736247. [CrossRef] [PubMed] [PubMed Central]
22. Karhanova, M.; Kovar, R.; Frysak, Z.; Sin, M.; Zapletalova, J.; Rehak, J.; Herman, M. Correlation between magnetic resonance imaging and ultrasound measurements of eye muscle thickness in thyroid-associated orbitopathy. *Biomed. Pap. Med. Fac. Univ. Palacky. Olomouc Czech Repub.* **2015**, *159*, 307–312. [CrossRef] [PubMed]
23. Fledelius, H.C.; Zimmermann-Belsing, T.; Feldt-Rasmussen, U. Ultrasonically measured horizontal eye muscle thickness in thyroid associated orbitopathy: Cross-sectional and longitudinal aspects in a Danish series. *Acta Ophthalmol. Scand.* **2003**, *81*, 143–150. [CrossRef] [PubMed]
24. Szelog, J.; Swanson, H.; Sniegowski, M.C.; Lyon, D.B. Thyroid Eye Disease. *Mo. Med.* **2022**, *119*, 343–350. [PubMed] [PubMed Central]
25. Yang, J.; Chen, J.; Shi, B.; You, Y.; Pi, X.; Zhao, G.; Jiang, F. Effects of various extraocular muscle enlargement patterns on muscle diameter index in graves ophthalmopathy patients: A retrospective cohort study. *Sci. Rep.* **2023**, *13*, 16939. [CrossRef] [PubMed] [PubMed Central]
26. Bharadwaj, S.R.; Hoenig, M.P.; Sivaramakrishnan, V.C.; Karthikeyan, B.; Simonian, D.; Mau, K.; Rastani, S.; Schor, C.M. Variation of binocular-vertical fusion amplitude with convergence. *Investig. Ophthalmol. Vis. Sci.* **2007**, *48*, 1592–1600. [CrossRef] [PubMed] [PubMed Central]
27. Perros, P.; Crombie, A.L.; Matthews, J.N.; Kendall-Taylor, P. Age and gender influence the severity of thyroid-associated ophthalmopathy: A study of 101 patients attending a combined thyroid-eye clinic. *Clin. Endocrinol.* **1993**, *38*, 367–372. [CrossRef] [PubMed]
28. Hesarghatta Shyamasunder, A.; Abraham, P. Measuring TSH receptor antibody to influence treatment choices in Graves' disease. *Clin. Endocrinol.* **2017**, *86*, 652–657. [CrossRef] [PubMed]
29. Nicolì, F.; Lanzolla, G.; Mantuano, M.; Ionni, I.; Mazzi, B.; Leo, M.; Sframeli, A.; Posarelli, C.; Maglionico, M.N.; Figus, M.; et al. Correlation between serum anti-TSH receptor autoantibodies (TRAbs) and the clinical feature of Graves' orbitopathy. *J. Endocrinol. Investig.* **2021**, *44*, 581–585. [CrossRef] [PubMed]
30. Tabasum, A.; Khan, I.; Taylor, P.; Das, G.; Okosieme, O.E. Thyroid antibody-negative euthyroid Graves' ophthalmopathy. *Endocrinol. Diabetes Metab. Case Rep.* **2016**, *2016*, 160008. [CrossRef] [PubMed] [PubMed Central]
31. Bartalena, L.; Kahaly, G.J.; Baldeschi, L.; Dayan, C.M.; Eckstein, A.; Marcocci, C.; Marinò, M.; Vaidya, B.; Wiersinga, W.M.; EUGOGO. The 2021 European Group on Graves' orbitopathy (EUGOGO) clinical practice guidelines for the medical management of Graves' orbitopathy. *Eur. J. Endocrinol.* **2021**, *185*, G43–G67. [CrossRef] [PubMed]
32. Lanzolla, G.; Marinò, M.; Marcocci, C. Selenium in the Treatment of Graves' Hyperthyroidism and Eye Disease. *Front. Endocrinol.* **2021**, *11*, 608428. [CrossRef] [PubMed] [PubMed Central]
33. Kang, H. Sample size determination and power analysis using the G*Power software. *J. Educ. Eval. Health Prof.* **2021**, *18*, 17. [CrossRef] [PubMed] [PubMed Central]

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Intraocular Pressure Measurements in Standing, Sitting, Prone, and Supine Positions

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Abstract: In this study, intraocular pressure (IOP) was measured in sitting, supine, prone, and standing (ST) positions and again five minutes after standing (ST-5) utilizing a Tono-Pen AVIA in 124 eyes of 62 healthy subjects with ages ranging from 21 to 59 years (mean 30 ± 10 years). In each subject, the average IOP of both eyes was used for the statistical evaluation. The mean IOP difference between the ST and sitting positions was −0.13 ± 1.63 mmHg (p = 0.548); between ST-5 and sitting, it was 0.53 ± 1.24 mmHg (p = 0.001); between supine and sitting, it was 1.30 ± 1.48 mmHg (p < 0.001); between ST and supine, it was −1.43 ± 1.74 mmHg (p < 0.001); between ST-5 and supine, it was −0.77 ± 1.59 mmHg (p < 0.001); between prone and supine, it was 2.24 ± 1.92 mmHg (p < 0.001); between ST and ST-5, it was −0.67 ± 1.84 mmHg (range: −7.5 to 5 mmHg) (p = 0.007); between prone and ST, it was 3.46 ± 2.01 mmHg (p < 0.001); between ST-5 and prone, it was −2.46 ± 1.67 mmHg (p < 0.001); and between sitting and prone, it was −3.22 ± 1.56 mmHg (p < 0.001). The results show a significant IOP increase in the ST-5 position, suggesting that such measurements need to be performed in an attempt to explain the progression of glaucoma in apparently normal-tension patients.

Keywords: intraocular pressure; hand-held tonometer; standing position; supine position; sitting position; normal-tension glaucoma

1. Introduction

The increase in intraocular pressure (IOP) is a well-established risk factor for glaucoma development and progression [1–3]. Given improvements in understanding glaucoma pathophysiology, IOP remains the only variable that therapy can improve [4]. For this reason, IOP measurement is part of routine ophthalmic examinations. Unfortunately, these examinations represent only a snapshot of the real IOP due to its circadian fluctuations that may influence glaucoma progression [5–7].

Among the variables that can influence this fluctuation, the position of the body can play a major role in nocturnal IOP elevation; in fact, several studies have demonstrated that the IOP may rise significantly when the patient assumes a supine position compared to a sitting position [8–10]. In some cases, this could explain why glaucomatous damage continues to progress despite the detection of a low IOP during routine ophthalmic examinations, suggesting that some high-risk patients should sleep with their head elevated [11]. It should be taken in account that many people can spend hours in a standing position during the day, and for this reason, it could be helpful to know if pressure changes occur in this situation. In a PubMed literature search, it was possible to find just four papers that reported some information about this topic [12–15]. Among these, in one paper, IOP measurements taken in a standing position were compared with those taken an unusual position, suspending the patients by the ankles from a gravity inversion device [12], while another study was performed on a very limited number of patients [13], and two others used rebound tonometers (Icare Pro–ICP) [14,15].

The rebound principle upon which Icare is based is different from the applanation principle of Goldmann applanation tonometry (GAT), which is still considered the gold

standard in IOP measurement. Also, Tono-Pen AVIA (TPA) uses the same physical principle to measure the IOP, but it has two advantages:

(1) The applanated area is much smaller, because the transducer tip has a diameter of 1.0 mm.
(2) TPA is portable and it does not need to be installed on a slit-lamp, and therefore it can be used to measure IOP in different IOP positions [16].

A previous paper published by De Bernardo et al. evaluated TPA and rebound tonometers in different body positions, but they carried out their study on a small number patients and not all body positions were analyzed [16]. On these bases, in continuity with previously published evidence, the aim of this study was to evaluate IOP measurements performed with an applanation-based tonometer by checking if similar changes were observed in a larger population after 5 min or more in a standing position and in a prone position.

2. Materials and Methods

One hundred and twenty-four eyes of 62 healthy subjects (16 males) with an age ranging from 21 to 59 years old (mean 30 $\pm$ 10 years) and a refractive error ranging from -6.50 to +1.25 D (mean -2.07 ± 2.13 D) were included in this prospective observational study.

The study was conducted in adherence to the tenets of the World Medical Association's Declaration of Helsinki. The purpose of the study was explained to all participants, who provided written informed consent. The study was approved by the Institutional Review Board of the University of Salerno (Cometico Campania Sud, Naples., Italy, Protocol No. 16544). Each subject underwent a general physical checkup to rule out any systemic disease.

The participants underwent a comprehensive ophthalmic examination that included the following:

- Best corrected visual acuity;
- Refractive error, performed with an objective method and perfected with a subjective method;
- Fundus examination;
- Axial eye length (AL), evaluated with an IOLMaster (Zeiss, Jena, Germany, version 5.4.4.00006);
- Central corneal thickness (CCT) measured with a Pentacam HR (Oculus, Wetzlar, Germany, version 1.19r11). IOP measurements were taken with TPA (Reichert Inc., Depew, NY, USA).

IOP was measured between ten and twelve a.m. by a single observer. TPA used the same physical principle as GAT to measure IOP, but the applanated area is much smaller. Before each measurement, the eyes were anesthetized with oxibuprocaine eye drops and the TPA probe tip was covered with a new latex tip cover. When ten valid readings were obtained by slightly touching the central cornea, the mean IOP readings were automatically averaged by the instrument. The measurement was shown on the liquid crystal display, situated on the device side, together with the "statistical confidence indicator", indicating that the standard deviation of the valid measurements was 5% or less of the number shown: the higher the value, the more reliable the measurements [16]. Only values higher than 90 were accepted.

The measurements were obtained in a sitting position, a supine position after five minutes of lying down, a prone position, a standing (ST) position, and after five minutes in a standing position (ST-5) in a random sequence [14]. The measurements in a prone position were taken with the patient's head protruding beyond the bed, with the chin resting on the bed and the eyes looking downwards. Unfortunately, not all patients were able to position their body to perform correct IOP measurements in a prone position: for this reason, prone measurements were taken in 23 patients. In addition, in 4 patients, the IOP measurements in an ST position were not performed. In each patient, the average of both eyes' IOP was analyzed using the paired Student's *t*-test. Single pairwise comparisons through T-tests were preferred to repeated-measures ANOVA tests because of the different sample sizes available. Correlations analyses were performed with Pearson's test

(r: correlation coefficient according to Pearson). All statistical analyses were performed with Microsoft Excel software, and they were double-checked with SPSS software (Version 26.0 IBM Inc., Chicago, IL, USA). Data were expressed as means ± standard deviations and p values < 0.050 was considered significant. The preliminary required sample size was computed with G*Power software (Version 3.1.9.7) by using the T test family option, two-tailed. Given an effect size dz of 0.786, determined with G*Power software, it was estimated that a sample size of 20 subjects would be necessary, with a significance level of 0.05 and a test power of 0.90.

3. Results

IOP measurements in the different positions (Table 1) and the statistical differences summarized in Figures 1–9 and Table 2 clearly show that:

- In the ST-5 and supine positions, the IOP is higher than in the sitting position ($p < 0.001$) in both cases (Figures 1 and 2, correspondingly);
- In the prone position, the IOP is higher than in the supine position ($p < 0.001$, Figure 3);
- In the supine position, the IOP is higher than in the ST position ($p < 0.001$, Figure 4), and this difference decreases with increasing time (ST-5 min, Figure 5);
- In the ST-5 min position, the IOP is higher than in the ST position ($p = 0.007$, Figure 6).

Table 1. Intraocular pressure measurements (in mmHg) in the different positions with a Tono-Pen Avia tonometer.

	Sitting	Supine	ST *	ST-5	Prone **
Mean	14.63	15.93	14.38	15.16	18.15
SD	2.82	3.27	2.96	3.18	3.19
Min	9.50	10.00	9.50	10.00	13.00
Max	22.50	25.50	24.00	24.00	23.00

ST: measurements obtained immediately after assuming a standing position; ST-5: measurements obtained after five minutes in a standing position; SD: standard deviation. * ST data are avilable only for 58 patients; ** prone position data are available only for 23 patients.

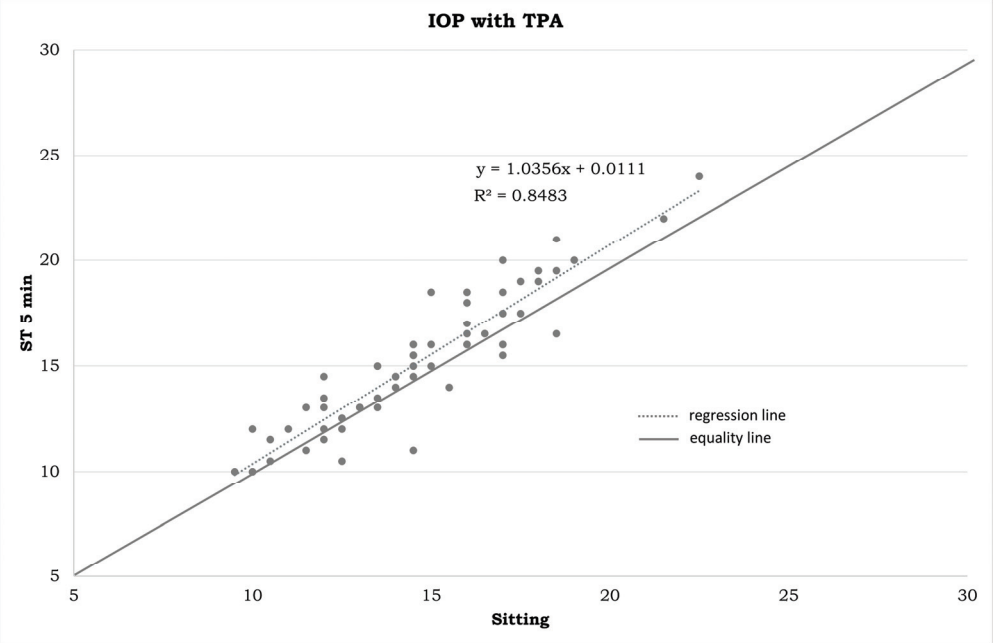

Figure 1. Plot between the IOP measurements in a sitting position on the horizontal axis and IOP measurements in the ST-5 min position on the vertical axis (in mmHg). IOP: intraocular pressure. TPA: Tono-Pen AVIA. ST-5 min: measurements obtained after five minutes in a standing position.

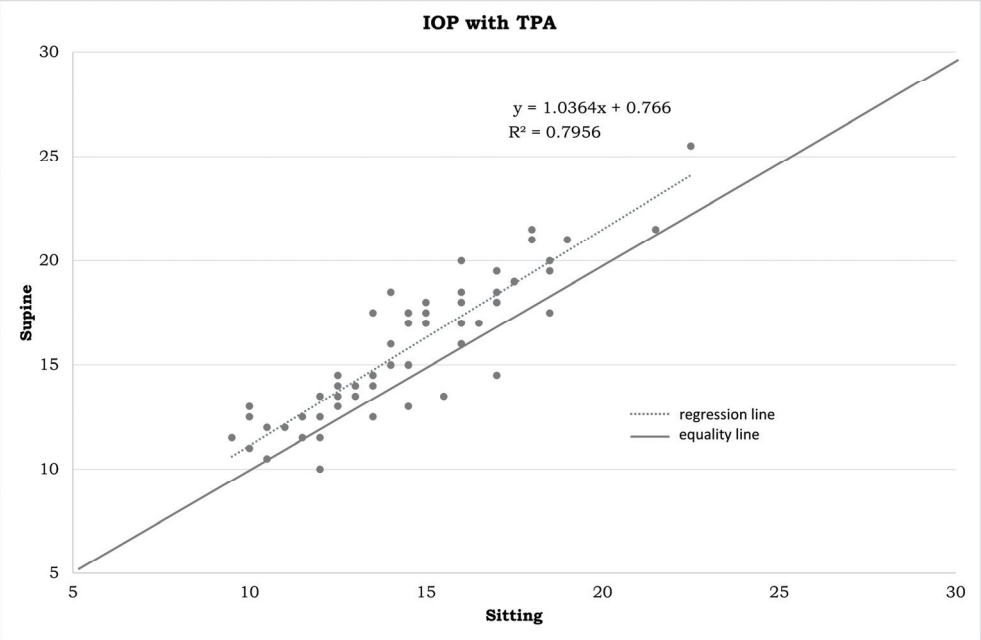

Figure 2. Plot between the IOP measurements in a sitting position on the horizontal axis and IOP measurements in a supine position on the vertical axis (in mmHg). IOP: intraocular pressure. TPA: Tono-Pen AVIA.

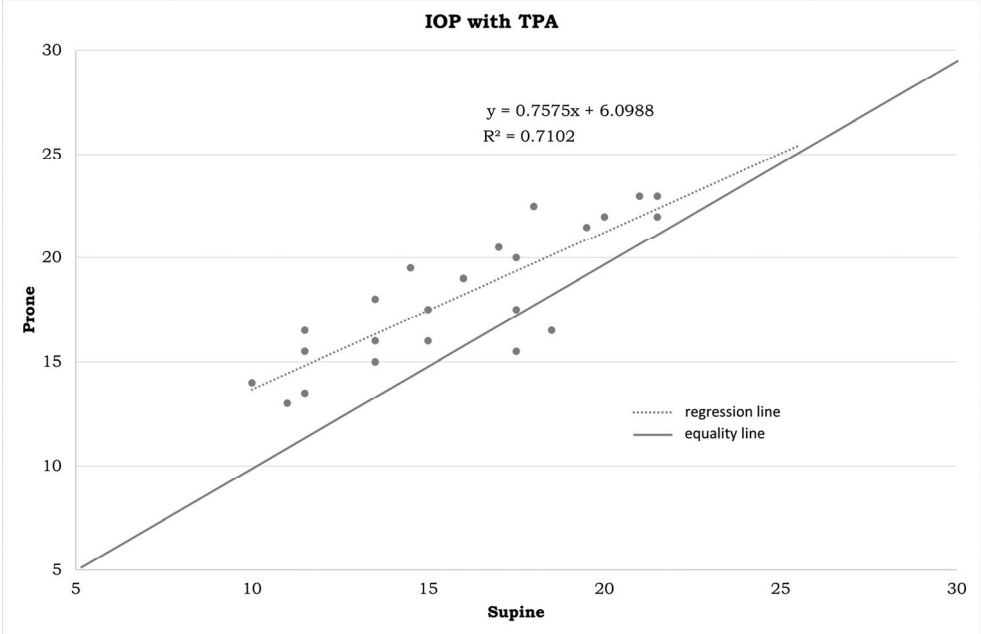

Figure 3. Plot between the IOP measurements in a supine position on the horizontal axis and IOP measurements in a prone position on the vertical axis (in mmHg). IOP: intraocular pressure. TPA: Tono-Pen AVIA.

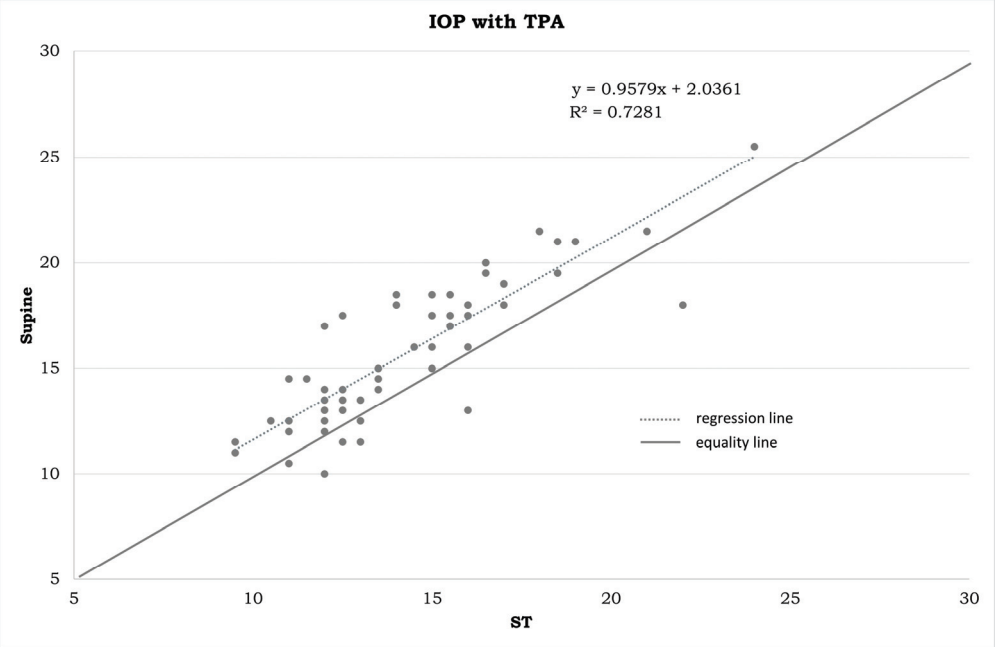

Figure 4. Plot between the IOP measurements in an ST position on the horizontal axis and IOP measurements in a supine position on the vertical axis (in mmHg). IOP: intraocular pressure. TPA: Tono-Pen AVIA. ST: measurements obtained immediately after assuming a standing position.

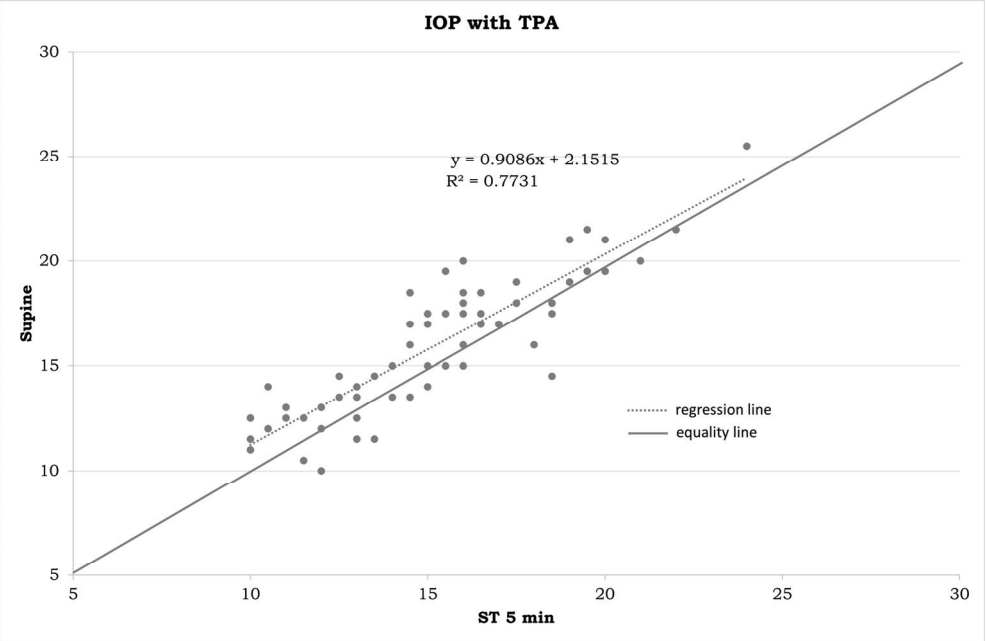

Figure 5. Plot between the IOP measurements in the ST-5 min position on the horizontal axis and IOP measurements in a supine position on the vertical axis (in mmHg). IOP: intraocular pressure. TPA: Tono-Pen AVIA. ST-5 min: measurements obtained after five minutes in a standing position.

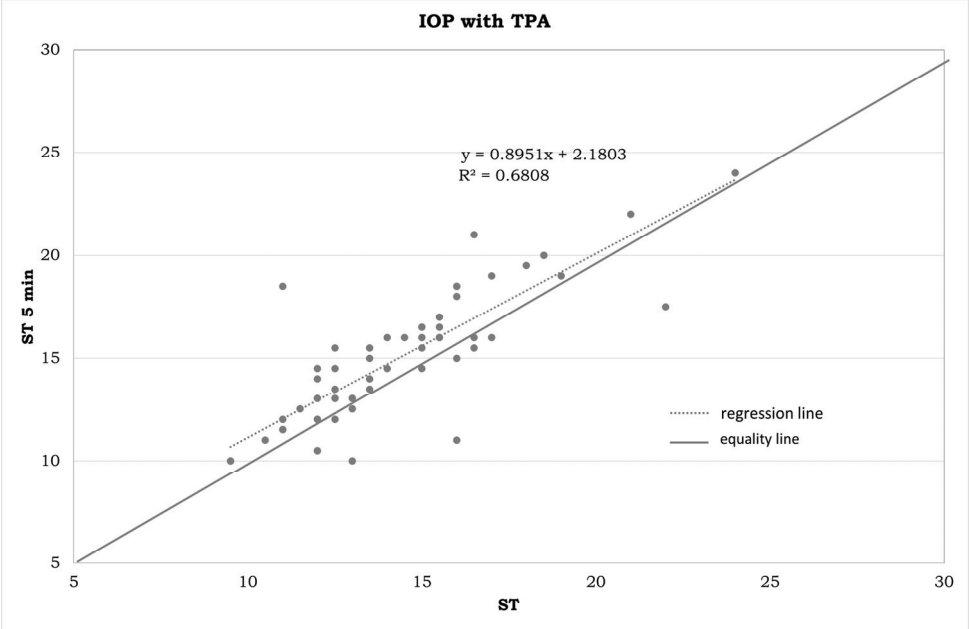

Figure 6. Plot between the IOP measurements in a ST position on the horizontal axis and IOP measurements in the ST-5 min position on the vertical axis (in mmHg). IOP: intraocular pressure. TPA: Tono-Pen AVIA. ST-5 min: measurements obtained after five minutes in a standing position. ST: measurements obtained immediately after assuming a standing position.

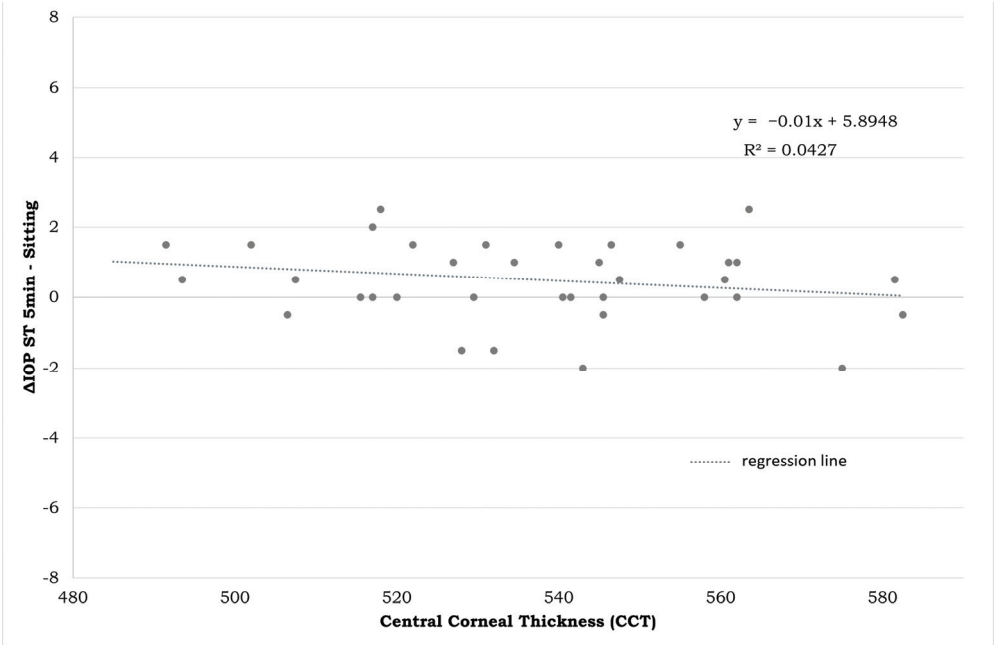

Figure 7. Plot between the CCT mean of both eyes of each subject on the horizontal axis (in micron) and difference in IOP measurements between the ST-5 and sitting positions on the vertical axis (in mmHg). CCT: central corneal thickness. IOP: intraocular pressure. ST-5 min: measurements obtained after five minutes in a standing position.

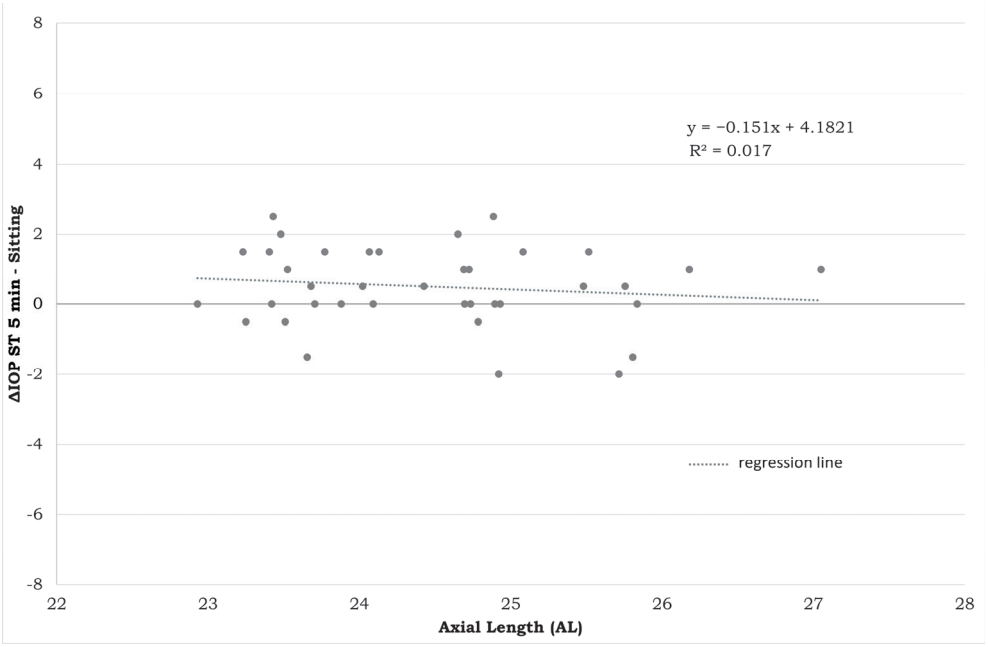

Figure 8. Plot between the AL mean of both eyes of each subject on the horizontal axis (in mm) and difference in IOP measurements between the ST-5 and sitting positions on the vertical axis (in mmHg). IOP: intraocular pressure. ST-5 min: measurements obtained after five minutes in a standing position.

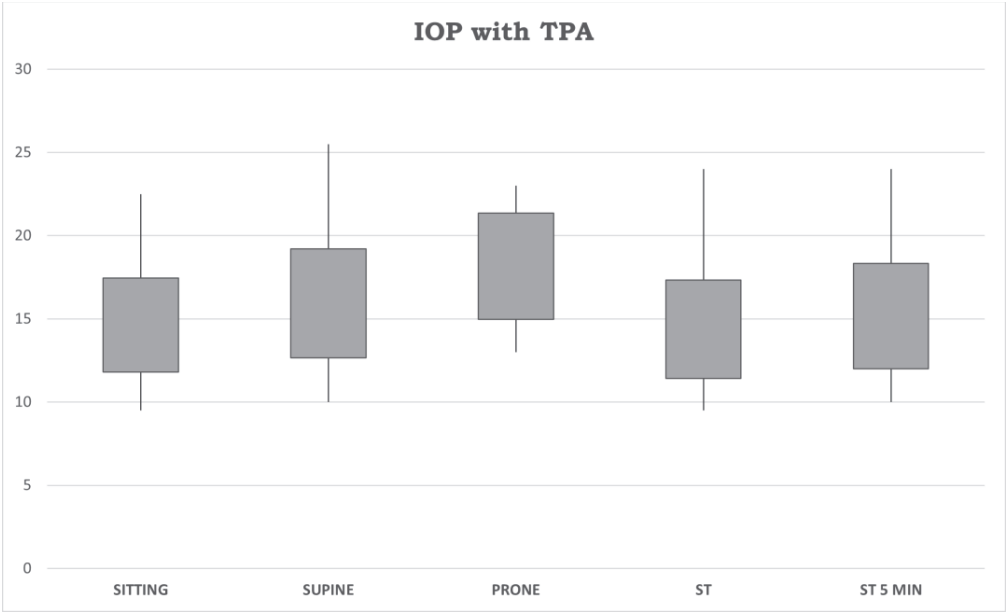

Figure 9. Standard deviations and minimum/maximum values for IOP measurements in the sitting, supine, prone, ST and ST-5 min positions (in mmHg). IOP: intraocular pressure. TPA: Tono-Pen AVIA. ST 5 MIN: measurements obtained after five minutes in a standing position. ST: measurements obtained immediately after assuming a standing position.

Table 2. Intraocular pressure difference (in mmHg) measured with a Tono-Pen AVIA tonometer between different positions, with statistical differences.

	Difference ST/Sitting	Difference ST-5/Sitting	Difference Supine/Sitting	Difference ST/Supine	Difference ST-5/Supine
Mean	−0.13	0.53	1.30	−1.43	−0.77
SD	1.63	1.24	1.48	1.74	1.59
95%CI	−0.56/0.30	0.22/0.85	−0.19/1.68	−1.89/−0.97	−1.16/−0.36
p	0.548	0.001	<0.001	<0.001	<0.001
	Difference Prone/Supine	**Difference ST/ST-5**	**Difference Prone/ST**	**Difference ST-5/Prone**	**Difference Sitting/Prone**
Mean	2.24	−0.67	3.46	−2.46	−3.22
SD	1.92	1.84	2.01	1.67	1.56
95%CI	1.41/3.06	−1.16/−0.19	2.59/4.33	−3.18/−1.73	−3.89/−2.54
p	<0.001	0.007	<0.001	<0.001	<0.001

ST: measurements obtained immediately after assuming a standing position; ST-5 min: measurements obtained after five minutes in a standing position; SD: standard deviation; 95%CI: 95% confidence interval around the mean; *p*: level of significance of paired *t*-test.

The CCT measured with the Pentacam HR was 535 ± 32 μm (range: 452 to 598 μm).

The AL obtained from the IOLMaster was 24.47 ± 1.28 mm (range: 21.70 to 27.16 mm).

IOP differences in the sitting and ST-5 min positions presented no correlation with CCT (Figure 7) and AL (Figure 8).

Means and standard deviations for IOP measurements in the sitting, supine, prone, ST, and ST-5 min positions (in mmHg) are shown in Figure 9.

Figures and Tables

Table 1 synthesizes the IOP values obtained with the TPA in all positions, while Table 2 shows the mean differences between IOPs measured in all positions, with the level of significance. We should reiterate that comparisons with ST data were performed based on 58 patients; meanwhile, analysis with prone position data were carried out on 23 patients.

Figures 1–6 represent the correlation analysis between IOP values in different body positions. A strong positive correlation was found

- between sitting and ST-5 IOP (r = 0.921);
- between sitting and supine IOP (r = 0.891);
- between supine and prone IOP (r = 0.843);
- between ST and supine IOP (r = 0.853);
- between ST-5 and supine IOP (r = 0.879);
- between ST and ST-5 IOP (r = 0.825).

For brevity, only the most clinically relevant correlations were reported.

The correlations between biometric parameters and IOP changes are shown in Figures 7 and 8. A low correlation rate was found between both AL and CCT and ΔIOP ST-5/sitting (r: −0.330 and r: −0.439, respectively), meaning that biometric parameters are less involved in IOP changes across different body positions. For brevity, only the most clinically relevant correlations were reported. Figure 9 reports graphically descriptive statistic data of IOP values in different body positions.

A cluster analysis based on patients' age was carried out in order to verify personalized responses. Due to the low number of prone measurements, these data were not considered in cluster analysis to avoid a too low sample size. The median age of subjects (25 years) was considered as the cut-off value for age range analysis. Overall, 35 subjects (31 in standing position) were evaluated in subgroup A (patients' age ≤ 25 years), while 27 subjects were evaluated in subgroup B (patients' age > 25 year). The results are reported in Table 3.

Table 3. Intraocular pressure difference (in mmHg) measured with a Tono-Pen AVIA tonometer between different positions divided by patients' age, with statistical differences.

A	**Difference ST-5/Sitting**	**Difference ST-5/Supine**	**Difference ST/ST-5**	**Difference Supine/Sitting**	**Difference ST/Sitting**	**Difference ST/Supine**
Mean	0.26	−0.93	−0.79	1.19	−0.55	−1.73
SD	1.26	1.61	2.02	1.60	1.65	1.58
95%CI	−0.17/0.69	−1.48/−0.38	−1.53/−0.05	0.63/1.74	−1.15/0.06	−2.31/−1.15
p	0.237	0.002	0.037	<0.001	0.074	<0.001
B	**Difference ST-5/Sitting**	**Difference ST-5/Supine**	**Difference ST/ST-5**	**Difference Supine/Sitting**	**Difference ST/Sitting**	**Difference ST/Supine**
Mean	0.89	−0.56	−0.54	1.44	0.35	−1.09
SD	1.13	1.56	1.63	1.32	1.49	1.87
95%CI	0.44/134	−1.17/0.06	−1.18/0.11	0.92/1.97	−0.23/0.94	−1.83/−0.35
p	<0.001	0.075	0.100	<0.001	0.232	0.005

A: patients' age $\leq$ 25 years; B: patients' age > 25 years; ST: measurements obtained immediately after assuming a standing position; ST-5 min: measurements obtained after five minutes in a standing position; SD: standard deviation; 95%CI: 95% confidence interval around the mean; *p*: level of significance of paired *t*-test; in bold: similar level of significance compared to the general population (Table 2).

The same trend was noted both in the general population and in the age-divided subgroups for supine/sitting and ST/supine. The difference in ST/sitting was not statistically significant in all groups, and instead a lower difference for ST-5/sitting was found in younger patients and a lower difference for ST-5/supine and ST/ST-5 was found in older patients.

4. Discussion

Goldmann applanation tonometry (GAT) is considered to be the gold standard in IOP measurements, but unfortunately it has several limitations, including that it needs to be mounted on a slit lamp, and for this reason, it is almost impossible to use in patients in a supine or a standing position; for this reason, this study was conducted with TPA, which has been reported to give reliable measurements compared to GAT in a sitting position [17].

In a PubMed literature search, it was possible to find several papers where the IOP was measured in sitting and supine position with different devices, some with the Tonopen [18–22], some others with the Icare [23], others with both tonometers [24–26], and only one with TPA [24]. However, just four papers investigated the IOP in a standing position, and they made different comparisons [12,13], or the authors used an ICP [14,15].

LeMarr et al. [12], in a group of 26 young subjects, evaluated the IOP changes between a standing and a head-down position. They found an increase in IOP in the head-down position, which a very unusual position; moreover, no comparison was made with a sitting or supine position.

Singleton et al. [13] evaluated the changes in IOP with postural changes (supine, sitting, and standing) in just 11 normal subjects, comparing them to the changes that occur in several groups of patients with different kinds of autonomic dysfunction. In this very small group of normal subjects, they found that the changes in IOP with postural changes were not statistically significant.

Meanwhile, some studies have measured the IOP disparity between sitting and supine postures. Lee et al. [17] examined 19 healthy young Korean participants, comparing the IOP in a sitting and supine position with a Tonopen XL: they observed an increase of approximately 3 mmHg when moving from a sitting to a supine position.

In another study, Lee et al. [23] measured the IOP in 40 eyes of 20 healthy Korean subjects using an Icare tonometer and found a statistically significant mean increase in IOP ($p < 0.001$) of about 2 mmHg when shifting from a sitting to a supine position.

Moster et al. [20] measured the IOP in both a sitting and a supine position in 45 glaucoma patients and 46 healthy subjects with a Tonopen. In each group, the authors found a

statistically significant mean IOP increase from a sitting to a supine position, being higher in glaucomatous patients (2.3 mmHg) than in healthy subjects (1.2 mmHg).

Barkana et al. tested the IOP with a Tonopen XL in 19 healthy subjects [21], first sitting then lying for 15 ± 5 min and 45 ± 5 min in a supine position; the same authors in another study measured the IOP with a Tonopen XL in twenty-one eyes of 21 healthy subjects [24], first in a sitting position and then after 10 min in a supine position. In both studies, the authors found an increase in IOP from a sitting to a supine position; furthermore, in the first study, the IOP after 45 min in a supine position was less than that measured after 15 min, without statistically significant differences.

Schweier et al. [25], in 36 eyes of 36 healthy individuals, measured IOP with ICP, TPA, and GAT in a sitting position and 10 min after reclining. They found the IOP to be lower in the sitting position compared to the reclining position with both hand-held tonometers, and the mean difference was greater with TPA (1.8 mmHg) than with ICP (0.8 mmHg) [25].

Sobczak et al. [26], in 71 healthy subjects, found no statistically significant differences between IOP measurements in sitting and supine positions using an ICP tonometer.

Our results regarding the IOP changes in a standing position cannot be compared with the previously published papers by LeMarr [12] and Singleton [13], due to the lack of measurements in the sitting position in the first case and the limited number of patients in the second case.

The results of the present study agree with those published by De Bernardo et al. [14], indicating that utilizing the ICP revealed a non-significant increase in the ST position compared to the sitting position, followed by a statistically significant increase in the ST-5 min position compared to the ST position. Mayali et al. [15], on the other hand, using rebound tonometry (ICP), found no IOP differences in the standing position versus other body positions.

Compared with other papers that considered only measurements in sitting and supine positions, the present study, performed with TPA, confirms these findings. However, our finding of a significant increase in the IOP in a prolonged standing position could be an interesting new finding, even if it is less than the increase in a supine position.

The IOP increase in the supine position may have different explanations; in particular, some researchers have indicated that this is due to an increase, in the supine position, in the episcleral venous pressure [27]. Other authors suggest that this could be due to choroidal vascular engorgement caused by the redistribution of bodily fluids in the supine position [28].

The results displayed in Figures 7 and 8 clearly show that the IOP measured in the different postures is not related to the CCT or to the AL. The reason for the increase in prolonged standing position is not as obvious. One hypothesis could be that, even if the exact mechanism of the IOP regulation is unknown, it may be under systemic vascular control, through the autonomous nervous system, which contributes to maintaining relatively constant blood flow to the tissue despite perfusion pressure fluctuations. Local myogenic, metabolic, and circulating humoral factors are involved in the autoregulation process [13,29]. However, future studies correlating the postural changes in IOP with eventual changes in blood pressure, heart rate, ocular perfusion pressure, and peripheral vasoconstriction could be useful to better understand the reason for these IOP modifications.

Although the sample in our study is not representative for all age groups, previous studies suggest that the IOP change associated with different body positions is not age-related [15]; a cluster analysis based on patients' age was carried out in order to verify if the same trend in IOP changes persists in different age ranges. As reported in Table 3, younger subjects showed a lower difference between the ST-5 and sitting positions than the general population; on the other hand, differences between ST-5 and both the supine and ST positions were lower in older patients. A more valid vascular flow redistribution could explain the lower difference between the first (sitting position) and the last (ST-5 position) IOP measurements in younger patients. On the other hand, a more stable choroidal vascular engorgement could explain the lower differences between ST-5 and both the ST

and supine positions in older subjects. These findings could help in a personalized analysis of IOP changes in normal subjects and glaucomatous patients, according to their age range. Unfortunately, due to only male subjects being examined in this study, a gender-based analysis of IOP changes in different positions was inexecutable.

One criticism that could be applied to this study is that the TPA was used instead of the GAT, which, introduced in 1957 by Hans Goldmann, represents the gold standard in measuring IOP [30]. Unfortunately, it is well known that the measured IOP value is influenced by several corneal parameters, such as biomechanical properties, CCT [31–35], corneal irregularities [36], and corneal refractive surgery [37,38]. Albis-Donado et al. evaluated different external factors that can influence GAT reliability: CCT is crucial, but the authors also emphasized the role of altitude [32]. In fact, they found a higher risk of underestimating IOP not only with thinner corneas, but also with a higher altitude. These outcomes are valid for GAT; on the other hand, dynamic contour tonometry (DCT) seems to be less affected by external factors [32]. Chen et al. demonstrated that CCT influences IOP measured not only with GAT, but also with rebound tonometry and with non-contact tonometry (NCT) [34]; they observed that NCT was mostly influenced by CCT, while GAT was less dependent on CCT.

Regarding corneal abnormalities, Knauf et al. reported that IOP measurement is challenging at various stages of keratoconus (KC), depending on the corneal thickness and biomechanical properties [35]. These features are altered in KC eyes. For similar reasons, IOP is difficult after corneal refractive surgery: changes in corneal thickness and corneal curvature affect IOP measurement reliability with different instruments, and topical steroids used postoperatively may mask the incidence and severity of steroid-induced ocular hypertension in the short term [38].

Since the purpose of this study was to compare IOP measurements in different positions, and GAT cannot be used when a patient is unable to sit at a slit lamp (small children, bed-ridden persons) or in situations such as taking measurements in a standing or supine position, TPA was chosen, among the hand-held IOP measuring devices, because it is one of the most widely used, and so far has not been tested for such a purpose.

5. Conclusions

In conclusion, the results of this study confirm the importance of measuring IOP in a supine position, but more importantly suggest that such measurements should also be performed after five minutes in a standing position, as in some jobs (e.g., waiters, policemen, chefs. . .) people can spend a significant amount of time standing, and this information could be helpful in understanding some cases of optic nerve damage or glaucoma progression, despite an apparently normal IOP.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Cometico Campania Sud, Italy (protocol code n° 16544).

Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Heijl, A.; Leske, M.C.; Bengtsson, B.; Hyman, L.; Bengtsson, B.; Hussein, M.; Early Manifest Glaucoma Trial Group. Reduction of intraocular pressure and glaucoma progression: Results from the Early Manifest Glaucoma Trial. *Arch. Ophthalmol.* **2002**, *89*, 1268–1279. [CrossRef] [PubMed]
2. Kass, M.A.; Heuer, D.K.; Higginbotham, E.J.; Johnson, C.A.; Keltner, J.L.; Miller, J.P.; Parrish, R.K.; Wilson, M.R.; Gordon, M.O.; Ocular Hypertension Treatment Study Group. The Ocular Hypertension Treatment Study: A randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma. *Arch. Ophthalmol.* **2002**, *120*, 701–713. [CrossRef] [PubMed]
3. Leske, M.C.; Heijl, A.; Hussein, M.; Bengtsson, B.; Hyman, L.; Komaroff, E.; Early Manifest Glaucoma Trial Group. Factors for glaucoma progression and the effect of treatment: The Early Manifest Glaucoma Trial. *Arch. Ophthalmol.* **2003**, *121*, 48–56. [CrossRef] [PubMed]
4. Weinreb, R.N.; Aung, T.; Medeiros, F.A. The Pathophysiology and Treatment of Glaucoma: A Review. *JAMA* **2014**, *311*, 1901–1911. [CrossRef] [PubMed]
5. Asrani, S.; Zeimer, R.; Wilensky, J.; Gieser, D.; Vitale, S.; Lindenmuth, K. Large diurnal fluctuations in intraocular pressure are an independent risk factor in patients with glaucoma. *J. Glaucoma* **2000**, *9*, 134–142. [CrossRef] [PubMed]
6. Caprioli, J. Intraocular pressure fluctuation: An independent risk factor for glaucoma? *Arch. Ophthalmol.* **2007**, *125*, 1124–1125. [CrossRef] [PubMed]
7. Caprioli, J.; Coleman, A.L. Intraocular pressure fluctuation: A risk factor for visual field progression at low intraocular pressures in the Advanced Glaucoma Intervention Study. *Ophthalmology* **2008**, *115*, 1123–1129. [CrossRef] [PubMed]
8. Tarkkanen, A.; Leikola, J. Postural variations of the intraocular pressure as measured with the Mackay-Marg tonometer. *Acta Ophthalmol.* **1967**, *45*, 569–575. [CrossRef] [PubMed]
9. Jain, M.R.; Marmion, V.J. Rapid pneumatic and Mackey-Marg applanation tonometry to evaluate the postural effect on intraocular pressure. *Br. J. Ophthalmol.* **1976**, *60*, 687–693. [CrossRef]
10. Krieglstein, G.; Langham, M.E. Influence of body position on the intraocular pressure of normal and glaucomatous eyes. *Ophthalmologica* **1975**, *171*, 132–145. [CrossRef]
11. Buys, Y.M.; Alasbali, T.; Jin, Y.P.; Smith, M.; Gouws, P.; Geffen, N.; Flanagan, J.G.; Shapiro, C.M.; Trope, G.E. Effect of sleeping in a head-up position on intraocular pressure in patients with glaucoma. *Ophthalmology* **2010**, *117*, 1348–1351. [CrossRef] [PubMed]
12. LeMarr, J.D.; Golding, L.A.; Adler, J.G. Intraocular pressure response to inversion. *Am. J. Optom. Physiol. Opt.* **1984**, *61*, 679–682. [CrossRef] [PubMed]
13. Singleton, C.D.; Robertson, D.; Byrne, D.W.; Joos, K.M. Effect of posture on blood and intraocular pressures in multiple system atrophy, pure autonomic failure, and baroreflex failure. *Circulation* **2003**, *108*, 2349–2354. [CrossRef] [PubMed]
14. De Bernardo, M.; Borrelli, M.; Cembalo, G.; Rosa, N. Intraocular Pressure Measurements in Standing Position with a Rebound Tonometer. *Medicina* **2019**, *55*, 701. [CrossRef] [PubMed]
15. Mayalı, H.; Tekin, B.; Kayıkçıoğlu, Ö.R.; Kurt, E.; İlker, S.S. Evaluation of the Effect of Body Position on Intraocular Pressure Measured with Rebound Tonometer. *Turk J. Ophthalmol.* **2019**, *49*, 6–9. [CrossRef] [PubMed]
16. De Bernardo, M.; Abbinante, G.; Borrelli, M.; Di Stasi, M.; Cione, F.; Rosa, N. Intraocular Pressure Measurements in Standing, Sitting, and Supine Position: Comparison between Tono-Pen Avia and Icare Pro Tonometers. *J. Clin. Med.* **2022**, *11*, 6234. [CrossRef] [PubMed]
17. Berk, T.A.; Yang, P.T.; Chan, C.C. Prospective comparative analysis of 4 different intraocular pressure measurement techniques and their effects on pressure readings. *J. Glaucoma* **2017**, *26*, 194–195. [CrossRef] [PubMed]
18. Lee, J.Y.; Yoo, C.; Jung, J.H.; Hwang, Y.H.; Kim, Y.Y. The effect of lateral decubitus position on intraocular pressure in healthy young subjects. *Acta Ophthalmol* **2012**, *90*, e68–e72. [CrossRef] [PubMed]
19. Fogagnolo, P.; Orzalesi, N.; Ferreras, A.; Rossetti, L. The circadian curve of intraocular pressure: Can we estimate its characteristics during office hours? *Investig. Ophthalmol. Vis. Sci.* **2009**, *50*, 2209–2215. [CrossRef]
20. Moster, S.J.; Fakhraie, G.; Venketesh, R.; Moster, M.L.; Zhao, Y.; Moster, M.R. Relationship of central corneal thickness to postural IOP changes in patients with and without glaucoma in southern India. *Int. Ophthalmol.* **2012**, *32*, 307–311. [CrossRef]
21. Barkana, Y. Postural change in intraocular pressure: A comparison of measurement with a Goldmann tonometer, Tonopen XL, Pneumatonometer, and HA-2. *J. Glaucoma* **2014**, *23*, e23–e28. [CrossRef] [PubMed]
22. Huang, R.; Ge, J.; Chen, G.; Gao, X.; Laties, A.M.; Zhang, X. Four measures of intraocular pressure fluctuation: Which correlates most optimally with actual office-hour readings? *J. Glaucoma* **2015**, *24*, 550–555. [CrossRef] [PubMed]
23. Lee, T.E.; Yoo, C.; Kim, Y.Y. Effects of different sleeping postures on intraocular pressure and ocular perfusion pressure in healthy young subjects. *Ophthalmology* **2013**, *120*, 1565–1570. [CrossRef] [PubMed]
24. Barkana, Y.; Gutfreund, S. Measurement of the difference in intraocular pressure between the sitting and lying body positions in healthy subjects: Direct comparison of the Icare Pro with the Goldmann applanation tonometer, Pneumatonometer and Tonopen XL. *Clin. Exp. Ophthalmol.* **2014**, *42*, 608–614. [CrossRef] [PubMed]
25. Schweier, C.; Hanson, J.V.; Funk, J.; Töteberg-Harms, M. Repeatability of intraocular pressure measurements with Icare PRO rebound, Tono-Pen AVIA, and Goldmann tonometers in sitting and reclining positions. *BMC Ophthalmol.* **2013**, *13*, 44. [CrossRef] [PubMed]

26. Sobczak, M.; Asejczyk, M.; Geniusz, M. Does body position, age, and heart rate induce IOP's changes? *Eur. J. Ophthalmol.* **2022**, *32*, 1530–1537. [CrossRef] [PubMed]
27. Sultan, M.; Blondeau, P. Episcleral venous pressure in younger and older subjects in the sitting and supine positions. *J. Glaucoma* **2003**, *12*, 370–373. [CrossRef] [PubMed]
28. Malihi, M.; Sit, A.J. Effect of head and body position on intraocular pressure. *Ophthalmology* **2012**, *119*, 987–991. [CrossRef] [PubMed]
29. Cowley, A.W.; Franchini, K.G. Neurogenic control of blood vessels. In *Primer on the Autonomic Nervous System*; Robertson, D., Low, P.A., Polinsky, R.J., Eds.; Academic Press: New York, NY, USA, 1996; pp. 49–55.
30. Whitacre, M.M.; Stein, R. Sources of error with use of Goldmann-type tonometers. *Surv. Ophthalmol.* **1993**, *38*, 1–30. [CrossRef]
31. Rosa, N.; De Bernardo, M.; Pepe, A.; Vitiello, L.; Marotta, G.; Imparato, R.; Capasso, L. Corneal thickness evaluation in healthy eyes: Comparison between two different Scheimpflug devices. *PLoS ONE* **2020**, *15*, e0243370. [CrossRef]
32. Albis-Donado, O.; Ramirez-Neria, P.; Rios-Acosta, N.; Stalmans, I. The influence of altitude on the differences between Goldmann tonometry and Pascal dynamic contour tonometry: An ecological meta-analysis. *Indian J. Ophthalmol.* **2024**, *72* (Suppl. 3), S398–S403. [CrossRef] [PubMed]
33. De Bernardo, M.; Vitiello, L.; Abbinante, G.; De Pascale, I.; Capasso, L.; Marotta, G.; Rosa, N. Comparison between two devices in the detection of corneal thickness changes after cataract surgery. *Sci. Rep.* **2021**, *11*, 6709. [CrossRef] [PubMed]
34. Chen, M.; Zhang, L.; Xu, J.; Chen, X.; Gu, Y.; Ren, Y.; Wang, K. Comparability of three intraocular pressure measurement: iCare pro rebound, non-contact and Goldmann applanation tonometry in different IOP group. *BMC Ophthalmol.* **2019**, *19*, 225. [CrossRef] [PubMed]
35. De Bernardo, M.; Casaburi, C.; De Pascale, I.; Capasso, L.; Cione, F.; Rosa, N. Comparison between dynamic contour tonometry and Goldmann applanation tonometry correcting equations. *Sci. Rep.* **2022**, *12*, 20190. [CrossRef] [PubMed]
36. Knauf, D.; Seitz, B.; Schießl, G.; Zemova, E.; Flockerzi, E. Analysis of Various Modalities for Intraocular Pressure Measurement in Relation to Keratoconus Severity in 246 Eyes of the Homburg Keratoconus Center. *Cornea* **2023**, *42*, 829–836. [CrossRef] [PubMed]
37. De Bernardo, M.; Capasso, L.; Caliendo, L.; Vosa, Y.; Rosa, N. Intraocular pressure evaluation after myopic refractive surgery: A comparison of methods in 121 eyes. *Semin. Ophthalmol.* **2016**, *31*, 233–242. [CrossRef]
38. Ang, R.E.T.; Bargas, N.V.R.; Martinez, G.H.A.; Sosuan, G.M.N.; Nabor-Umali, M.I. Comparison of Three Tonometers in Measuring Intraocular Pressure in Eyes That Underwent Myopic Laser in situ Keratomileusis and Photorefractive Keratectomy. *Clin. Ophthalmol.* **2022**, *16*, 1623–1637. [CrossRef]

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Review

Ocular Manifestations and Complications of Patent Foramen Ovale: A Narrative Review

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Abstract: Patent foramen ovale (PFO) is a prevalent congenital cardiac anomaly associated with a persistent opening between the atrial septum, allowing communication between the left and right atria. Despite often being asymptomatic, PFO can lead to various clinical presentations, including cryptogenic stroke and other embolic events. Transient visual disturbances, alterations in the visual field, migraine with aura, impaired eye movement and endogenous eye infections may prompt patients to seek ophthalmological consultation. Understanding these diverse clinical scenarios is crucial for early detection, appropriate management and mitigating the morbidity burden associated with PFO. This narrative review aims at examining the spectrum of clinical presentations of ocular pictures associated with PFO. The pathophysiology, diagnosis and treatment methods for PFO will be described, emphasizing the importance of a multidisciplinary approach involving ophthalmologists, cardiologists, neurologists and imaging specialists. In the future, prospective studies and clinical trials are warranted to provide further insights into the preventive role and optimal therapeutic strategies for managing PFO-related ocular complications, ultimately guiding clinical decision making and optimizing patient care.

Keywords: patent foramen ovale; eye; eye disorders; retinal artery occlusion; migraine with aura; impaired eye movement

1. Introduction

Patent foramen ovale (PFO) is a common congenital cardiac anomaly characterized by a persistent opening between the atrial septum, which allows for communication between the left and right atria [1]. After birth, changes in blood pressure lead to the closure of the foramen ovale in about 75% of newborns. As a result, its prevalence is estimated to be around 25–30% in the general population [2–4]. This defect creates a potential pathway for paradoxical embolism, allowing venous thrombi or other embolic material to bypass pulmonary circulation and enter systemic circulation, leading to deleterious clinical consequences [5].

Despite often being asymptomatic, PFO has been associated with various clinical presentations, ranging from cryptogenic stroke to different embolic events [5,6]. To highlight the impact of PFO, patients with cryptogenic stroke have a 2.9 times higher likelihood of having it compared to controls [7]. PFO may be implicated in approximately two-thirds of cryptogenic stroke cases and potentially up to 80% in younger patients [8]. In addition to the well-known and extensively studied complications documented in the literature, more potential clinical presentations have been linked to PFO. These presentations may encompass heterogeneous, less common, yet clinically relevant symptoms that may prompt patients to seek ophthalmic consultation. These symptoms could include transient visual disturbances, alterations in the visual field, migraine and other oculomotor signs that raise suspicion of possible PFO-related complications [9,10]. Several studies and case

reports have described acute retinal ischemic events, including transient visual loss and retinal artery occlusions. Understanding the potential clinical manifestations of PFO is crucial for early detection and appropriate management, particularly considering the highly significant prevalence of this condition. Recognizing these manifestations early can facilitate prompt referral to cardiology, appropriate diagnostic work-up and consideration for available PFO closure treatment [11].

In this review, we will analyze the spectrum of clinical presentations associated with PFO, primarily focusing on manifestations that may drive patients to ophthalmological evaluation, also trying to highlight the importance of mitigating the morbidity burden associated with PFO, given its high prevalence in the general population.

2. Materials and Methods

We utilized the PubMed medical database for our search. The term "patent foramen ovale" was combined with the words "eye", "eye disorders", "eye diseases", "retina", "ophthalmology", "eye palsy", "migraine with aura". The text keywords were chosen using information from pertinent bibliographies and the latest literature. The earliest publishing date of the search was set for January 1990, and it ended in March 2024.

Only English-language articles and reviews were analyzed, even though no language limits were placed on the searches. Moreover, manual searches were conducted within initial findings to identify further bibliographic references.

A total of 801 full-text articles were identified on PubMed, 679 of which were excluded after the first screening. The remaining 122 articles were evaluated for eligibility. After a full-text evaluation, 73 papers were retained as relevant articles and were used in this review.

3. Results

3.1. Patent Foramen Ovale: Pathophysiology, Diagnosis and Screening

PFO is derived from embryological folding misplacement. Briefly, the septum primum originates from the upper part of the atrium. The septum secundum forms through a folding of the atrial walls, creating the ostium secundum, which serves as a passage for oxygenated blood to flow from right to left during fetal life. A PFO occurs when the septum primum and septum secundum remain unjoined near the anterior and superior edge of the oval fossa. PFO size in adults is heterogeneous, ranging from 1 mm to 15–19 mm [4]. Of note, larger size and specific morphologies have been shown to correlate with enhanced risk of cerebrovascular accidents [12,13].

Usually, the diagnosis of PFO is made incidentally during routine imaging studies or after the patient presents with a potential clinical manifestation, prompting further imaging evaluation. The diagnosis is pursued through multimodality ultrasound imaging, including transthoracic echocardiography (TTE), transesophageal echocardiography (TEE) and transcranial Doppler using aerated saline as a contrast agent. TTE is often the initial screening tool, providing valuable information about the cardiac structure and function. However, its sensitivity in detecting PFO may be limited due to technical factors and the position of the septum, probably leading to minimal shunting miss [14,15]. Conversely, TEE offers higher sensitivity and specificity that reaches 100% when a contrast agent is used [16,17], allowing for closer visualization of the interatrial septum and providing detailed images of the fossa ovalis properly describing the PFO size and morphology [17]. It is particularly useful in cases in which TTE results are inconclusive or when a higher level of detail is required. Additionally, TEE enables the assessment of associated cardiac anomalies and facilitates the guidance of percutaneous closure procedures. Complementing echocardiographic techniques, by monitoring cerebral blood flow, transcranial Doppler can detect microembolic signals arising from right-to-left shunting through the PFO, especially when contrast injection is performed before Valsalva maneuver [18]. Its sensitivity was shown to be heterogeneous compared to intracardiac imaging [19–22]. However, these imaging modalities together play a crucial role in the comprehensive evaluation and management of patients with suspected PFO.

Screening for a PFO should be selectively conducted among individuals who have recently experienced clinical events potentially suggestive of PFO presence and who stand to benefit from PFO treatment. TEE is indicated in patients with a higher likelihood of having a PFO, for example, when assessing young individuals with unexplained strokes or any other plausible symptom linked to PFO; for patients with a lower probability of PFO, contrast TTE or transcranial Doppler may serve as reasonable initial diagnostic methods.

3.2. PFO and Acute Retinal Ischemic Events

Transient monocular vision loss (TMVL)/amaurosis fugax (AF), central retinal artery occlusion (CRAO), branch retinal artery occlusion (BRAO) and cilioretinal artery occlusion (CLRAO) are acute retinal ischemic events manifesting as sudden, painless monocular visual losses with different degrees of severity. The occlusion of the retinal artery can be transient or permanent and may result in impaired blood flow in the ocular circulation, which is associated with a high cardiovascular morbidity and mortality risk [23].

TMVL/AF is a condition characterized by a transient episode of monocular blindness that typically lasts for seconds to minutes. It occurs due to a temporary interruption of blood flow at the level of the ophthalmic artery or central retinal artery and its branches. The most common cause of TMVL/AF in the elderly population is embolism, characterized by a small clot or debris traveling through the bloodstream and blocking a blood vessel. These emboli can originate from various sites, including the carotid arteries, heart or other blood vessels [24]. Although in younger people, TMVL/AF is often associated with temporary spasm at the level of ocular circulation, monocular TMVL/AF may be the consequence of cardiogenic emboli secondary to cardiac malformations. Accordingly, in a recent cohort study, 15% of patients with TMVL/AF were found to have a PFO [25].

CRAO is a disease in which the main artery supplying blood to the retina becomes occluded. It manifests as an acute loss of vision, typically resulting in visual acuity of 20/400 or poorer; its incidence is 1 in 100,000 individuals, with a mean age of presentation of 60 years (Figure 1A–C) [26].

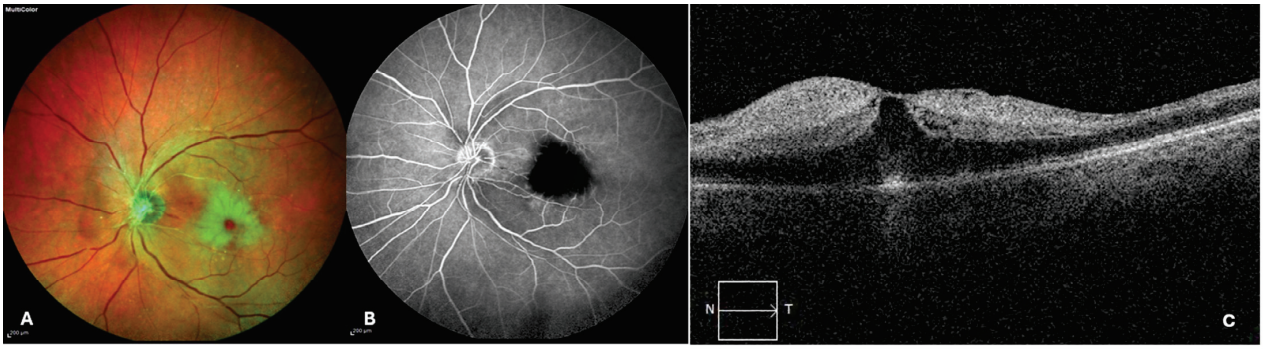

Figure 1. Retinal ischemic events related to PFO: (**A**) Multimodal imaging of a CRAO. On fundus photography, the retina at the posterior pole appears pale and opaque due to the ischemia; a distinctive cherry-red spot is visible at the fovea, which remains red due to the underlying choroidal circulation. The retinal arteries are thin and narrowed. (**B**) Fluorescein angiography reveals a hypofluorescent macular spot secondary to the ischemia of retinal layers. (**C**) The latter is confirmed by optical coherence tomography (OCT), which shows hyper-reflective and edematous inner retinal layers.

BRAO is a disorder in which one of the branches of the central retinal artery becomes blocked, leading to restricted blood flow to a portion of the retina; its incidence is closer to 5 per 100,000 persons per year [27]. The symptoms of BRAO can vary depending on the severity and location of the occlusion, but usually, the prognosis is better than in CRAO, resulting in a localized scotoma or in a sectoral visual field defect. Both of these ischemic disorders can be linked to PFO [28].

Another distinct clinical entity is the CLRAO. The cilioretinal artery arises from the short posterior ciliary vessels, and its prevalence ranges from 6.9% to 49.5%. It may play

a role in providing additional blood supply to the macular region of the retina, thus preserving central vision, in cases where the main retinal vasculature is compromised, such as in CRAO [29]. CLRAO can be a consequence of a hemodynamic blockage of the artery caused by a rise in intraluminal pressure in retinal vasculature exceeding the level of perfusion pressure, or it can be secondary to embolism or thrombosis, or it can recognize an arteritic etiology. Symptoms of CLRAO may include sudden blurring, scotoma or distortion of central vision. Since CLRAO has been linked to PFO through an embolic mechanism, the presence of an underlying cardiac defect should be excluded in young people presenting this disorder [28,30,31].

The diagnosis of acute retinal ischemic events is clinical and based on different possible morphologic findings detectable on fundus examination, such as retinal afferent pupillary defect (RAPD), retinal whitening, macular cherry-red spot and intra-arterial emboli. Retinal fluorescein angiography, optical coherence tomography (OCT) and OCT angiography (OCTA) can confirm the diagnosis, showing delayed arterial filling, retinal ischemia and hyper-reflective inner retinal layers [31–33]. Therapeutical approaches, including anterior chamber paracentesis, drug-lowering intraocular pressure and ocular massage, are often useless [34].

Despite being rare among younger patients, retinal arterial obstructions are described to be linked to underlying cardiac abnormalities in 45% of individuals below the age of 45 [35]. Numerous reports have detailed the connection between PFO and retinal ischemic disorders [25,28,30–33,36–51] (Table 1). In a cohort of 18 patients diagnosed with either central or branch retinal artery occlusion, TEE was conducted to probe potential underlying cardiac or aortic disorders as sources of retinal emboli. The investigation unveiled that 17% of the patients exhibited a PFO [48]. Fekri and colleagues analyzed 23 cases of retinal ischemic events in the presence of PFO, revealing a comparable incidence of BRAO and CRAO. Most cases were under 50 years old (78.3%), and roughly 50% of patients underwent uncomplicated PFO closure. TEE exhibited higher sensitivity than TTE in diagnosing the underlying cardiac disease (71.4% versus 28.6%) [28]. Similar findings were reported by Wieder et al., demonstrating that TEE detected PFO in 85.7% of cases compared to 14.3% with TTE in the context of ischemic retinal disorders in patients with a mean age of 42.4 years [32].

Table 1. Summary of the studies showing retinal ischemic events in patients with PFO.

Author (Year)	Reference	Type of Study	Population	Outcomes
Sverdlichenko et al. (2024)	[25]	Prospective cohort study	20 cases under 45 years of age with TMVL (4 men and 16 women)	All patients included were referred for neuroimaging and cardiac investigations. Two of the thirteen patients who completed echocardiography had PFO (15%).
Fekri et al. (2024)	[28]	Case-study-based review	23 cases of CRAO (n = 10), BRAO (n = 10) and CILRAO (n = 3) with PFO	A total of 78.3% of the cases were under 50 years of age. A high RoPE score ($\geq$7, suggestive of paradoxical embolism via PFO) was estimated for 71.4% of patients. TEE exhibited higher sensitivity than TTE in diagnosing the underlying cardiac disease (71.4% versus 28.6%). Approximately half of the patients underwent percutaneous closure of the PFO with no complications.
Jurgens et al. (2022)	[31]	Review and case report	1 case	A pregnant woman developed cilioretinal artery occlusion of thromboembolic origin associated with PFO. OCTA enabled risk-free imaging of retinal vessel perfusion during pregnancy.
Wieder et al. (2021)	[32]	Review and case report	1 case	A 43- year-old man presented with CRAO in the setting of PFO. TEE appeared more sensitive than TTE in diagnosing PFO (85.7% versus 14.3%).
Chatziralli et al. (2015)	[33]	Case report	1 case	A 35-year-old man presented with a left inferior BRAO. Cardiologic examination with TEE revealed a right-to-left shunt across PFO. The patient developed no other embolic event at the 9-month follow-up, having undergone an operation for PFO repair.

Table 1. *Cont.*

Author (Year)	Reference	Type of Study	Population	Outcomes
Inatomi et al. (2001)	[36]	Retrospective study	22 cases	A total of 22 patients with retinal artery occlusion (RAO) were analyzed. TEE findings were abnormal in 13 (59%) of the 22 patients. PFO was observed in 9% of patients.
Tayyab et al. (2023)	[37]	Case report	1 case	A teenage girl presented with CRAO in the right eye, which was accompanied by frontal headaches and vertigo. Investigations to rule out hematologic, vascular and cardiac causes were performed. TEE revealed PFO as the cause of this cryptogenic stroke.
Ho and Spaide (2007)	[38]	Case report	1 case	A healthy 15-year-old boy developed a CRAO with cilioretinal artery sparing after a fractured clavicle. Initially, no abnormalities were found after extensive systemic examinations. Subsequently, PFO was revealed by TEE, and the patient was scheduled for cardiac surgery.
Gabrielian et al. (2009)	[39]	Case report	1 case	A healthy 17-year-old male presented with sudden, painless loss of vision in his right eye while playing basketball. Fundus examination and fluorescein angiography confirmed a retinal artery occlusion. The hematological/infectious work-up, including TTE, was negative. However, TEE showed PFO. The patient underwent a successful percutaneous femoral catheterization to close the defect.
Nakagawa et al. (2004)	[40]	Case report	1 case	A 43-year-old woman presented a bilateral CRAO within one month. The patient was treated with urokinase and with an anticoagulant. The corrected VA recovered completely in the right eye and partially in the left eye. After extensive examination, TEE revealed a right-to-left shunt through PFO. The patient was treated with anticoagulants and has suffered no recurrence of CRAO.
Ascuitto et al. (2021)	[41]	Case report	1 case	A patient presented with painless, sudden visual loss in his left eye. He was diagnosed with CRAO, likely from a paradoxical embolus associated with an atrial septal aneurysm containing PFO. The patient underwent successful percutaneous closure of the PFO without complication.
Shoeibi et al. (2013)	[42]	Case report	1 case	A 29-year-old female patient presented a BRAO. The patient was treated with oral acetazolamide, topical timolol, ocular massage and anterior chamber paracentesis. The visual field defect partially recovered. After an extensive work-up, a small-sized PFO was detected by TEE. Low-dose aspirin therapy was initiated, and over the subsequent two years, no other embolic event occurred.
Wisotsky et al. (1993)	[43]	Case report	2 cases	A 28-year-old man and a 19-year-old woman presented BRAO. In each patient, PFO was demonstrated via transesophageal echocardiography after a transthoracic study disclosed no abnormalities.
Liu et al. (2017)	[44]	Case report	1 case	A 21-year-old man presented with an eight-day history of a blurry patch of vision in the right eye. A BRAO was diagnosed after fundus and OCT examinations. A TTE bubble contrast study showed a large right-to-left shunt at the atrial level. Percutaneous closure of the PFO was undertaken. Normal visual acuity after 12 months was recovered.
Grudzińska et al. (2021)	[45]	Case report	1 case	A 33-year-old Caucasian man presented a superior nasal loss of visual field and central scotoma in his left eye. A BRAO was diagnosed. The patient underwent standard TTE without any pathological findings. TEE revealed a marked leak through PFO, and cardiologists decided on foramen ovale closure.

Table 1. *Cont.*

Author (Year)	Reference	Type of Study	Population	Outcomes
Sheth et al. (2009)	[46]	Case report	1 case	An 18-year-old male claimed sudden-onset left visual loss. Ophthalmological examination revealed an afferent pupillary defect secondary to left hemiretinal artery occlusion. A subsequent cardiology review with TEE revealed PFO, which was closed surgically.
Sabanis et al. (2020)	[47]	Case report	1 case	A patient with no significant comorbidities experienced a paradoxical thromboembolic episode of CRAO. After a comprehensive examination, PFO was diagnosed after a bubble contrast TTE.
Kramer et al. (2001)	[48]	Retrospective observational case series	18 cases	A total of 18 patients who were diagnosed with retinal artery occlusion (7 CRAO, 11 BRAO) were analyzed. Cardiac or thoracic aortic pathologic conditions, which were a possible source of the retinal emboli, were detected by TEE in 13 of the 18 patients (72%). They included aortic arch atheroma (n = 7), mitral annulus calcification (n = 4), left atrial appendage thrombus (n = 2), valvular abnormalities (n = 5), left atrial smoke (n = 3) and PFO (n = 3). TEE showed higher sensitivity than TTE in detecting the cardiac emboli source.
Zhu et al. (2021)	[49]	Case report	1 case	A 46-year-old woman presented an amaurosis fugax lasting 20 min and a persistent visual alteration in her left eye. Fundus examination did not reveal significant abnormalities. Fundus fluorescein angiography showed a long arm–retina circulation time. Imaging examinations revealed bilateral internal carotid artery (ICA) hypoplasia and a variation in the circle of Willis. Furthermore, TEE showed the presence of a PFO and a cardiogenic embolic event. Aspirin 100 mg/day was given to the patient, and PFO occlusion surgery was recommended.
Si et al. (2023)	[50]	Review and case report	1 case	A 40-year-old woman reported a gradual decline in visual acuity, with visual field defects that had developed in two stages after HA injection as facial filler into the nasal root. Multiple retinal artery occlusions were diagnosed. PFO was detected by TEE. The patient was treated with intravenous dexamethasone and cobamamide, as well as extracorporeal counterpulsation therapy. Visual acuity improved from counting fingers at 30 cm to 20/133 within 3 days.
Pipolo et al. (2023)	[51]	Case report	1 case	A 28-year-old woman presented at the clinic 20 h after an acute visual field defect in the right eye. At that time, she was 18 weeks pregnant and had no report of complications in her previous pregnancy. After ophthalmological evaluation, BRAO was diagnosed. A transcranial Doppler with contrast indicated a right-to-left intracardiac shunt, confirmed by the presence of PFO at the TEE. Thrombophilic conditions were excluded. Enoxaparin 1 mg/kg was started and kept until the delivery. Then, a surgical closure of PFO was programmed.

While the exact pathogenesis of retinal arterial blockage in individuals with PFO remains incompletely understood, several mechanisms have been proposed. These include emboli formation within the atrial septum, transient arrhythmias leading to thrombus formation and paradoxical emboli originating from the peripheral venous system, which reach the head circulation occluding the ophthalmic artery and its branches [33,37].

Once diagnosed, considering PFO closure may be advisable to reduce the risk of additional ischemic vasculo-occlusive events [28,33,39,41,44–46].

Moreover, in addition to well-known risk factors like smoke and hypertension, an association with conditions such as internal carotid artery (ICA) hypoplasia, filler injections and pregnancy has also been described in patients with PFO developing retinal ischemic events [26,31,32,36,41,49–51].

ICA is one of the most important blood sources to the head, and its hypoplasia is rare. Many cases of abnormal development of ICA are asymptomatic, thanks to the compensatory action of collateral circulation. However, Zhu and co-authors reported a case of an apparently healthy woman who developed an AF due to the presence of a concomitant PFO in association with ICA hypoplasia that resulted in an embolism at the level of the retinal artery [49].

Facial filler with hyaluronic acid (HA) has previously been linked to visual loss. The retrograde flow mechanism is widely recognized as the primary explanation for sudden vision loss following HA injection for facial filler procedures. This phenomenon occurs when the injected filler material inadvertently enters a blood vessel, leading to its backward movement against the natural blood flow direction until it reaches the ocular circulation [52]. Nevertheless, in a recent report, a young woman presented multiple retinal artery ischemic events occurring in a peculiar pattern. Indeed, the patient's visual loss presented in two stages, rather than directly after injection, and was associated with dyspnea. Hence, the authors speculated that the HA entered systemic venous circulation, reached the right heart, passed directly into the left heart through a PFO and then caused retinal artery occlusion [50].

Various physiological adaptations, encompassing complex cardiovascular, coagulative, hormonal and immunological changes, occur during pregnancy and can trigger vascular occlusive events. The concomitant presence of PFO increases the risk of ocular thromboembolic events, as demonstrated by retinal vascular occlusion episodes described in two different reports. In these situations, OCTA, as a dye-free, non-invasive tool, represents a valuable option to diagnose and analyze the retinal perfusion status in pregnancy [31,51].

Therefore, PFO should be considered in cases of young patients who develop unexplained acute retinal ischemic events.

3.3. PFO and Migraine with Aura

Migraine is a neurological disorder characterized by recurrent, severe headaches, often accompanied by other symptoms, such as nausea, vomiting, sensitivity to light and visual disorder. Migraine attacks can cause significant pain and discomfort, sometimes lasting for hours to days, and can greatly disrupt daily life activities. Migraine with aura is a type of migraine characterized by specific neurological and visual symptoms, known as "aura", which usually occurs before the headache phase. The aura symptoms can vary widely among individuals but commonly include visual disturbances, such as flashing lights, blind spots or zigzag patterns which are usually bilateral. Other sensory disorders like tingling sensations in the face or hands, speech difficulties and confusion can be present. These symptoms develop gradually over a few minutes and last for about 20–60 min before the headache phase begins [53]. Multifactorial causative mechanisms, such as genetic, environmental and neurological factors, are recognized to be involved in migraine. Different triggers include stress, bright lights, certain foods, changes in sleep patterns, hormonal variations, sensory stimuli, weather changes and strong odors [53].

Migraine prevalence varies across different populations and regions, with a higher incidence among females [54]. The association between PFO and migraine has been widely described in the literature [10,55,56] (Table 2). Specifically, the relationship between PFO and migraine with aura was first described in a study carried out by Del Sette and colleagues. Among 80 migraineurs with aura, with an average age of 37.24 years, 36 individuals (45%) were found to have a cardiac right-to-left shunt [55]. Furthermore, migraine with aura appears to be more prevalent in individuals with PFO, and the latter is more common in migraineurs with aura than in the general population. Accordingly, a meta-analysis conducted by Schwedt et al. demonstrated a higher prevalence of PFO among migraine patients with aura (40.9–72.0%) compared to migraineurs without aura (16.2–33.7%). Moreover, the prevalence of migraine in individuals with PFO varied from 22.3% to 64.3% [10].

Table 2. Summary of the studies showing correlation between migraine and PFO.

Author (Year)	Reference	Type of Study	Population	Outcomes
Schwedt et al. (2008)	[10]	Systematic review	18 studies, 1517 patients	The aim of this study was to examine the prevalence of migraine in patients with PFO, the prevalence of PFO in migraineurs and the effect of PFO closure on migraine. The estimated strength of association between PFO and migraine, reflected by summary odds ratios (ORs), was 5.13 [95% confidence interval (CI) 4.67, 5.59], and between PFO and migraine with aura, the OR was 3.21 (95% CI 2.38, 4.17). The association between migraine and PFO was OR 2.54 (95% CI 2.01, 3.08). Six studies on PFO closure suggested improvement in migraine. The low-to-moderate grade of evidence from observational studies supports an apparent association between PFO and migraine. PFO closure seemed to affect migraine patterns favorably.
Del Sette et al. (2008)	[55]	Prospective study	87 patients	The objective of this study was to clarify the relationship between right-to-left shunt (RLS) and white matter lesions (WMLs) in patients with migraine with aura (MA). Among 80 migraineurs with aura, with an average age of 37.24 years, 36 individuals (45%) were found to have a cardiac right-to-left shunt. The comparison of T2-weighted MRI scans revealed no significant difference in the number of lesions or lesion loads between patients with and without RLS. In conclusion, RLS does not increase the likelihood of finding WMLs in migraineurs.
Liu et al. (2020)	[56]	Narrative review	99 articles	In this article, the potential pathophysiological mechanisms of migraine, the clinical symptoms of migraine with PFO and the clinical features of PFO with migraine were described. The incidence of PFO in migraine patients is higher than that in the general population, suggesting that PFO and migraine may be risk factors for each other, but more research is needed to confirm this speculation. An increasing number of studies have found that migraines with aura are more closely associated with PFO, and the presence of right-to-left shunt (RLS) increased the likelihood of aura attacks. The evidence supports a "dose–response" relationship between migraine and PFO, although more work needs to be conducted in terms of patient selection as well as the inclusion of an antiplatelet control group for PFO closure interventions to uncover possible beneficial results in clinical trials.
Wilmshurst (2018)	[57]	Randomized controlled trial	147 patients	The Migraine Intervention with STARFlex Technology (MIST) trial was a randomized double-blind trial in patients with severe intractable migraine with aura and moderate–large PFO that compared the implantation of STARFlex devices with the intention of closing their PFO versus a sham procedure. The results showed no significant difference in migraine outcomes between those receiving the STARFlex implant and those in the sham group.
Dowson et al. (2008)	[58]	Randomized controlled trial	432 patients	The aim of this study was to investigate whether closing patent foramen ovale (PFO) could effectively treat migraine with aura. Patients were randomly assigned to transcatheter PFO closure with the STARFlex implant or to a sham procedure. No significant difference was observed in the primary endpoint of migraine headache cessation between the implant and sham groups.
Mattle et al. (2016)	[59]	Randomized controlled trial	107 patients	The Percutaneous Closure of Patent Foramen Ovale In Migraine with Aura (PRIMA) study aimed to evaluate the efficacy of percutaneous patent foramen ovale (PFO) closure in reducing migraine symptoms in patients diagnosed with migraine with aura. Patients were divided in two groups and treated with a PFO closure or medical treatment. Despite the safety of the procedure, PFO closure did not reduce overall monthly migraine days.
Tobis et al. (2017)	[60]	Randomized controlled trial	230 patients	The Prospective, Randomized Investigation to Evaluate Incidence of Headache Reduction in Subjects With Migraine and PFO Using the AMPLATZER PFO Occluder to Medical Management (PREMIUM) study investigated migraine reduction in subjects with persistent migraines and PFO. It compared medical therapy alone to therapy combined with PFO closure using the Amplatzer device. The trial found no superior reduction in migraine attacks with PFO closure compared to sham control. However, it did show a significant decrease in headache days among subjects with or without aura following PFO closure.

Table 2. *Cont.*

Author (Year)	Reference	Type of Study	Population	Outcomes
Giardini et al. (2006)	[61]	Prospective study	38 patients	This study evaluated the long-term outcomes of transcatheter patent foramen ovale (PFO) closure on migraine headache with aura (MHA) and recurrent stroke risk. Among 38 patients followed for at least 3 years after the procedure, 92% reported complete resolution of MHA after an average of 4.9 years. Recurrent stroke occurred in 5.3% of cases within 5 years, predominantly within the first 15 months after the procedure. Overall, the MIDAS scores significantly decreased (from 38.6 $\pm$ 26.3 to 4.4 $\pm$ 5.1, $p < 0.0001$) following intervention.
Yan et al. (2024)	[62]	Retrospective study	78 patients	This study aimed to investigate the therapeutic efficacy of patent foramen ovale (PFO) closure in migraine patients with a massive right-to-left shunt (RLS). The study included 51 patients without white matter lesions (WMLs) (control group, CG) and 27 patients with white matter lesions (WMLs) (observation group, OG). PFO closure proved effective and safe in treating migraine patients with RLS. However, for those with WMLs, clinical attention should be directed toward the treatment of WMLs.
Elbadawi et al. (2018)	[63]	Meta-analysis	3 studies, 484 patients	The analysis included three key studies: the MIST, PRIMA and PREMIUM trials. The primary outcome demonstrated a significant reduction in monthly migraine attacks, favoring PFO closure over control groups ($p = 0.01$). Although complete resolution of migraine attacks showed a trend favoring PFO closure, the difference did not achieve statistical significance. In patients with predominant aura migraine attacks, PFO closure demonstrated a significant reduction in migraine attacks. No significant differences in responders' rate between PFO closure and control groups were found.
Shi et al. (2017)	[64]	Meta-analysis and systematic review	8 articles, 546 patients	The study investigated how the closure of patent foramen ovale (PFO) affected migraine in 546 patients, distinguishing between those with migraine with aura (MA) and migraine without aura (MwoA). Following PFO closure, migraine symptoms improved in 81% of MA patients and 63% of MwoA patients. The benefits of PFO closure were significantly greater for patients with MA compared to patients with MwoA ($p = 0.03$).
Zhang et al. (2022)	[65]	Meta-analysis	3 RCTs, 1 pooled study, 8 case series, 1165 patients	The aim of the study was to evaluate the impact of patent foramen ovale (PFO) closure on migraine. The findings revealed a statistically significant reduction in both monthly migraine attacks and migraine days, along with a substantial decrease in migraine frequency and severity in patients undergoing PFO closure, particularly those with migraines accompanied by aura (MA). The meta-analysis supports PFO closure as a potentially effective and safe therapeutic option for reducing the migraine burden, particularly in patients with MA.
Zhang et al. (2021)	[66]	Meta-analysis	3 RCTs, 4 observation studies, 887 patients	The meta-analysis findings indicate that PFO closure leads to a substantial reduction in both monthly migraine attacks and migraine days per month compared to control groups. This effect was particularly pronounced in patients with migraine accompanied by aura, where there was a notable increase in the rate of complete cessation of migraine attacks following the procedure. These results underscore the potential of PFO closure as an effective therapeutic option for managing migraine, especially in cases where traditional treatments have been insufficient.

The pathophysiology of migraine with aura in patients with PFO remains uncertain. Various vasoactive substances, including serotonin or 5-hydroxytryptamine, typically processed through the pulmonary circulation, may bypass this route via the PFO, potentially triggering migraines. Additionally, small emboli passing through the PFO into the arterial system could lead to microinfarctions or cortical spreading depression, precipitating migraine attacks. Furthermore, focal areas of reduced blood flow near the ischemic threshold in the occipital regions may lead to visual symptoms during the aura episode [56,57].

Although multiple studies have investigated the effect of closing PFO on migraine, the therapeutic effect of this surgical procedure is still controversial. The MIST trial found no significant difference between patients who underwent PFO closure and those who did not [58]. On the other hand, the PRIMA trial did not demonstrate a reduction in its primary

endpoint based on decreasing migraine days [59], while the PREMIUM trial showed a statistically significant decrease in headache days but failed to achieve a reduction in its primary outcome, defined as a responder rate with a 50% decrease in migraine attacks [60]. Conversely, other reports showed a significant decrease in the frequency of migraine attacks following PFO closure in patients with migraine with aura [61–66]. Specifically, an overall reduction in the frequency of migraine attacks per day and a decrease in the number of headache days were observed over one month [65,66]. Moreover, in patients whose migraine episodes were with aura, there was a decrease in migraine attacks following PFO closure compared to control groups [63].

Considering that visual symptoms usually precede the headache, ophthalmologists play an important role in diagnosing migraine with aura and differentiating it from other ophthalmic conditions that may present with similar symptoms, such as retinal ischemia or retinal detachment. Additionally, ophthalmologists may collaborate with neurologists in the management of migraine with aura, providing guidance on lifestyle modifications and strategies to manage migraine triggers (e.g., bright lights, stress and irregular sleep patterns) that may exacerbate symptoms. Moreover, they may prescribe medications to alleviate symptoms or recommend specialized examinations, such as visual field testing or neuroimaging studies, to further evaluate the underlying cause of symptoms [67].

3.4. PFO and Impaired Eye Movement

Eye movements are coordinated motions of the eyes, which allow perception of the surrounding space, the tracking of objects and focusing on specific targets. These movements are controlled by a complex system, involving muscles, nerves and various brain regions. Oculomotor disfunctions are characterized by the weakness or paralysis of the muscles within or surrounding the eye, resulting in impaired ocular action or control (Figure 2A,B). These can be caused by different factors, including nerve damage, trauma, inflammation, underlying systemic disorders or ischemic strokes. Injuries at the level of brain visual-related areas can impair the oculomotor system, resulting in several alterations in coordination, visual acuity and visual field orientation [68].

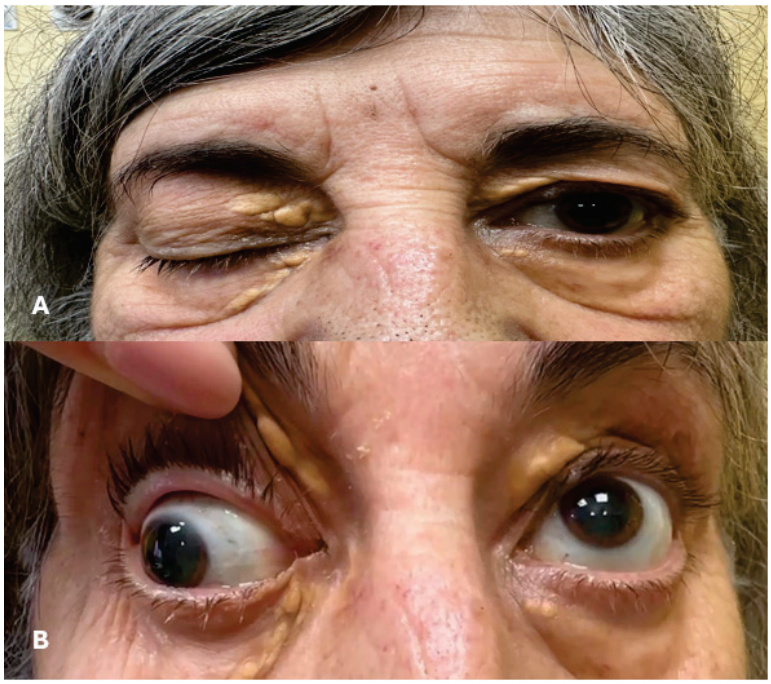

Figure 2. Impaired eye movement related to PFO: (**A**) Right eye third nerve palsy presenting a complete ptosis. (**B**) After eyelid elevation, the right eye appears to be positioned downward and outward, with inability to adduce, infraduce or overduce. The pupil is dilated with slow reaction.

The association between PFO and impaired eye movement is rare, and only few cases have been described [69–73] (Table 3). Cerebral lesions, involving the midbrain and other vision areas, were considered the consequence of paradoxical emboli resulting from the direct pass in the vascular system and in the cerebral circulation through the right–left shunt [69–73]. Internuclear ophthalmoplegia (IO)—a disorder characterized by a lesion of medial longitudinal fasciculus resulting in an ipsilesional adduction deficit with a nystagmus of the abducting eye—is often secondary to brain ischemia. A 39-year-old woman presented bilateral ptosis and IO associated with PFO diagnosed with TEE. After undergoing PFO closure and starting aspirin therapy for secondary prevention, the patient remained clinically stable over a one-year follow-up. [69]. A previously healthy 16-year-old girl experienced an IO secondary to a lesion of the left midbrain at the level of quadrigeminal lamina. Six months after aspirin therapy and PFO closure, her cerebral lesions and her eye movements were restored [70]. In addition, Zhuang and colleagues reported a case of a 55-year-old male who experienced a complete peripheral facial palsy and horizontal gaze palsy after Valsalva maneuver caused by paradoxical embolization from PFO. After 6 months and surgical closure, the symptoms improved [71]. Conversely, a 61-year-old man who had a severe embolic event involving the posterior cerebral artery territory, resulting in left hemiparesis, dysarthria, bilateral ptosis, and impaired eye movement on both sides, experienced only partial improvement after anticoagulant therapy. [72]. Khan described a case of an acute pupil-sparing complete third nerve palsy (complete ptosis, moderate hypotropia, large exotropia and no supraduction/infraduction/adduction) in a healthy young woman with PFO. After PFO closure, the neurological recovery was complete, except for diplopia and relatively comitant hypotropia, which responded well to conventional strabismus surgery [71]. Thus, despite only a few cases having been reported, PFO should be considered in cases of impaired eye movement secondary to embolic stroke in apparently healthy people [69–73].

Table 3. Summary of the articles showing correlation between impaired eye movement and PFO.

Author (Year)	Reference	Type of Study	Population	Outcomes
Xie et al. (2022)	[69]	Case report	1 case	A 39-year-old woman presented with sudden bilateral ptosis and diplopia exacerbated by leftward gaze, persisting for 3 days. Neurological examination and MRI confirmed acute midbrain infarction. The tests excluded other stroke causes but confirmed a large right-to-left shunt via PFO. She underwent PFO closure and received aspirin.
Mazurkiewicz-Bełdzińska et al. (2015)	[70]	Case report	1 case	A healthy 16-year-old girl had sudden diplopia and dizziness following a headache. MRI confirmed left midbrain ischemia with left internuclear ophthalmoplegia. PFO was discovered by TTE and TEE. Medical treatment led to full recovery of neurological deficits in two weeks. Six months after aspirin therapy and PFO closure, her cerebral lesions and her eye movements were restored.
Zhuang et al. (2021)	[71]	Case report	1 case	A 55-year-old man presented with sudden bilateral gaze palsy, upbeat nystagmus and facial paralysis. MRI revealed new pontine infarctions linked to PFO with significant shunt. He was treated with rivaroxaban and underwent PFO closure, resolving his symptoms. A follow-up MRI at 6 months showed no new lesions.
Takahashi et al. (2018)	[72]	Case report	1 case	A 61-year-old heavy smoker presented with sudden vision distortion and left leg weakness, revealing multiple brain infarctions correlated with PFO. Treatment included anticoagulants and antiplatelets, but severe residual deficits persisted after 12 weeks.
Khan (2012)	[73]	Case report	1 case	A healthy 23-year-old woman experienced sudden right third nerve palsy and facial weakness. MRI showed midbrain infarction linked to a large PFO. Surgical closure resolved the symptoms; strabismus surgery corrected residual right hypotropia, ensuring stable vision postoperatively.

3.5. PFO and Endogenous Eye Infections

Endophthalmitis refers to inflammation and infection of the fluids and tissues inside the eye and can result in devastating sight-threatening and systemic complications (Figure 3A–C). It can recognize an exogenous or endogenous origin. Most cases are of exogenous origin, typically arising from microbes entering the eye through an external source like penetrating trauma, intraocular surgery or corneal infection. Conversely, endogenous endophthalmitis results from the spread of bacterial or fungal infections from a distant site in the body, typically through the bloodstream [74]. In this type of endophthalmitis, the damage occurs mainly due to a septic embolus entering the posterior segment vasculature and acting as a trigger for the dissemination of micro-organisms into the surrounding tissues. After passing the blood–ocular barrier, the infection extends from the retina and choroid into the vitreous cavity, and subsequently, to the anterior chamber of the eye [75]. The common sources of infection leading to endogenous endophthalmitis include bacterial endocarditis, intravenous drug use and certain systemic infections like candidiasis or tuberculosis [74].

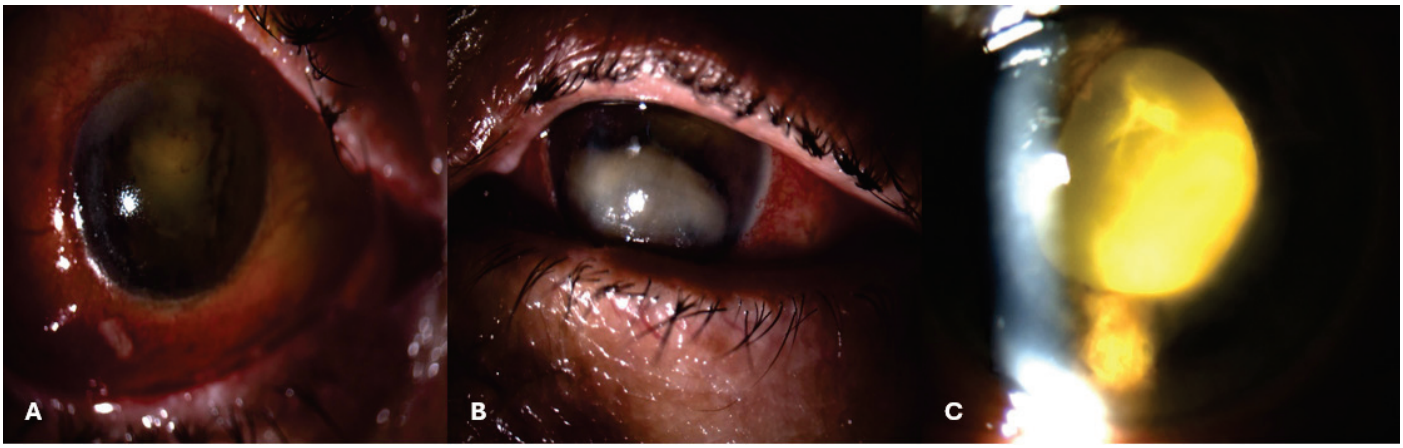

Figure 3. Endogenous eye infection related to PFO: (**A**–**C**) Slit lamp images of endophthalmitis showing conjunctival redness, purulent discharge, chemosis, hypopyon and fibrin in the pupillary field.

Rodrigues et al. described a case of endogenous endophthalmitis that developed as the first manifestation of right-sided endocarditis in a patient with an undiagnosed PFO [76]. A 66-year-old female with chronic renal failure presenting fever and eye endophthalmitis was treated with systemic antibiotics and enucleation. However, since a persistent systemic infection with positive blood cultures was observed, adjunctive analyses, including TEE, were performed, and a right endocarditis with a PFO was diagnosed [76]. Hence, even though paradoxical embolization through a PFO from right-sided endocarditis is rare [77], the presence of an interatrial defect caused ocular infection in this case. Therefore, an underlying cardiac defect should be taken into account in cases of endogenous endophthalmitis, where the source of primary infection is unidentified.

4. Discussion

The spectrum of clinical presentations associated with PFO encompasses a wide array of manifestations, including ophthalmological conditions like retinal artery occlusions, migraine with aura, impaired eye movements and endogenous infections (Figure 4).

The management of arterial occlusion remains a subject of ongoing debate. In the acute phase, the foremost goal for the ophthalmologist is to promptly restore retinal blood flow. Prolonged hypoxia has been demonstrated to lead to irreversible visual damage, underscoring the urgency of this intervention. Moving into the subacute phase, the ophthalmologist's focus shifts to assessing the patient in order to ascertain the underlying causative mechanism. This evaluation is crucial for preventing further ocular or systemic complications [34]. Despite the lack of clinical trials, the associations between PFO and acute retinal ischemic

events have been described in multiple reports. According to retinal and ophthalmic artery occlusions guidelines provided by the American Academy of Ophthalmology, conditions like ophthalmic artery occlusion, CRAO and BRAO can potentially indicate life-threatening situations [78]. Therefore, particularly in cases where the initial examination does not reveal a causative mechanism for embolic disease, considering the significant risk of ischemic stroke associated with ocular arterial occlusions, ophthalmologists must refer patients to a stroke center for multidisciplinary medical evaluation, including echocardiography and other imaging analyses.

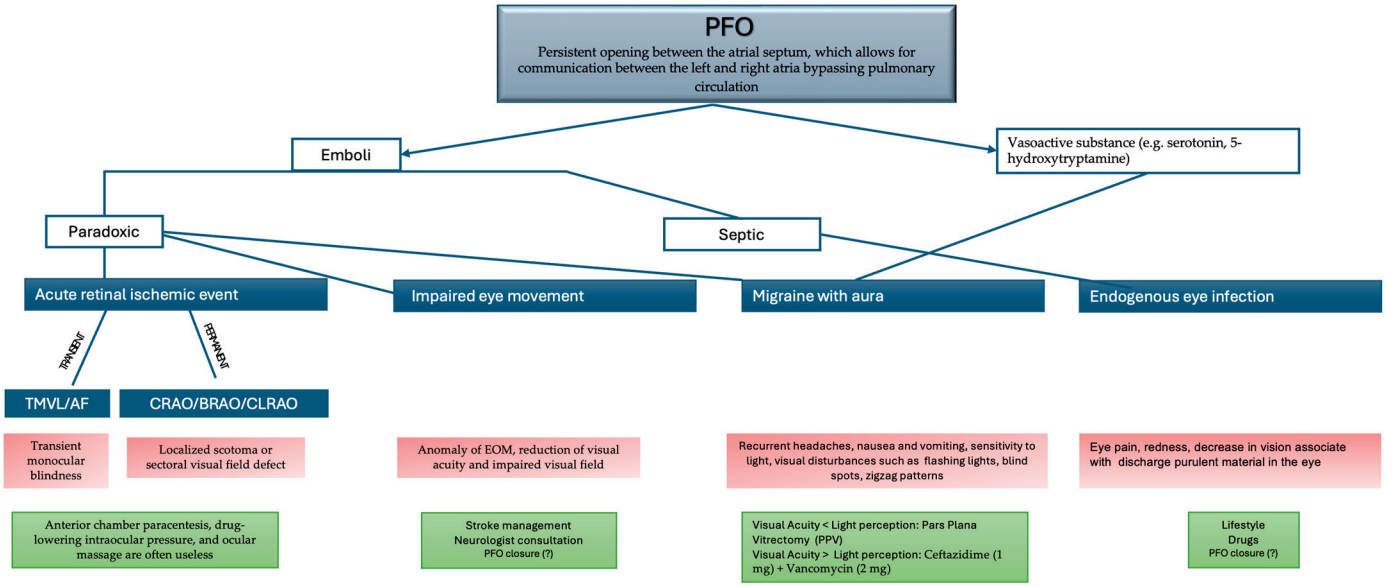

Figure 4. The diagram schematically illustrates the mechanism by which ophthalmic complications occur due to PFO. The symptoms of each condition are presented in the red squares, while the green squares show the possible management strategies.

Ophthalmologists frequently serve as the initial specialists to evaluate individuals experiencing visual symptoms related to migraines. The exact pathophysiological mechanisms linking PFO to migraine with aura remain uncertain. However, the higher prevalence of PFO among migraineurs with aura compared to the general population was shown in several studies, suggesting a relationship between these two entities. Although PFO closure has shown contrasting results in the management of migraine, considering the low complication rate of the procedure, surgical intervention, as secondary prevention, represents a valid option.

Managing ocular palsies typically involves several approaches, depending on the underlying cause, which need to be identified, and on the severity of the condition. Impaired eye movement in the presence of PFO is rarely reported. Nevertheless, in cases of cryptogenic embolic strokes affecting visual motility-related brain regions, the clinical suspicion of PFO should be raised, especially in young patients.

When suspecting endogenous endophthalmitis, an accurate diagnostic evaluation is crucial to identify a potential hematogenous spread of micro-organisms from a distant source in the body. It is worth noting that the presence of PFO may elevate the risk of paradoxical embolization. Indeed, particularly in patients with predisposing factors, such as bacterial endocarditis, endogenous ocular infection could be secondary to the presence of an undetected communication between the two atria. Despite ongoing debates, the consensus on the therapeutic approach to these conditions remains elusive. However, according to the guidelines from the European Society of Cataract and Refractive Surgery, pars plana vitrectomy appears to offer the most favorable visual outcomes in cases where visual acuity is limited to light perception. For patients with visual acuity exceeding light perception, the therapeutic strategies remain uncertain. Evidence suggests that combining

the same antibiotics via both intravitreal and systemic routes is the most effective approach. Administering ceftazidime (1 mg) and vancomycin (2 mg) separately is considered the gold standard [79].

In summary, after diagnosing an ocular embolic event, ophthalmologists should ideally require further investigation, including a TTE or a TEE, by echocardiographic imaging specialists. Once a PFO is diagnosed, potential interventional therapies should be considered, including PFO closure and medical therapy. The meta-analysis by Vukadinović et al. indicated that percutaneous PFO closure significantly reduces the risk of ischemic recurrences compared to medical therapy, especially in cases of large or moderate shunts [80]. Similarly, Turc et al. stated that PFO closure is superior to antithrombotic therapy in preventing stroke recurrence after cryptogenic stroke despite an increased risk of atrial fibrillation after the surgical procedure [81]. Conversely, Liu and colleagues reported that, compared with drug therapy, PFO closure reduces the risk of recurrent stroke and the incidence of serious bleeding without increasing the risk of new-onset atrial fibrillation or atrial flutter [82].

Hence, while the direct involvement of ophthalmologists in diagnosing PFO itself may be limited, their expertise in assessing ocular health and recognizing subtle signs of systemic diseases is invaluable. The correlation between ocular signs and symptoms and systemic diseases has been well documented. Recently, the interest in identifying ocular risk factors or biomarkers for diagnosing or excluding systemic diseases has significantly increased, leading to the emergence of oculomics. By examining the eye's structure and function, oculomics aims to provide insights into a patient's overall health, identifying the potential risk factors for diseases beyond the eye [83]. In this context, the deep-learning algorithm RetiCAC, which uses retinal photographs to predict the presence of coronary artery calcium (CAC), has demonstrated improved cardiovascular risk stratification compared to traditional clinical parameters [84].

Nonetheless, collaboration with other specialists like cardiologists and neurologists still remains essential for ensuring timely diagnosis, comprehensive evaluation and appropriate treatment selection.

5. Conclusions

It should be noted that PFO is a highly common condition that can cause severe ocular complications. This review analyzed the diverse clinical scenarios in which PFO may be implicated, shedding light on the importance of early recognition and multidisciplinary management in optimizing patient care. Ophthalmologists, as the first healthcare professionals to encounter patients presenting with ocular symptoms and signs, upon identifying suspicious findings, should be aware of this possible subtle entity and promptly refer patients to appropriate specialists.

6. Future Directions

Given the limited number of reports on certain ocular disorders associated with PFO, future prospective studies and clinical trials are warranted to provide further insights into the preventive role and optimal therapeutic strategies for managing these conditions. Large-scale, multicenter studies with long-term follow-up periods would help elucidate the natural history of PFO-related ocular complications. Additionally, effective research comparing different treatment modalities and their impact on clinical outcomes would be valuable for guiding clinical decision making and optimizing patient care.

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References

1. Homma, S.; Messé, S.R.; Rundek, T.; Sun, Y.-P.; Franke, J.; Davidson, K.; Sievert, H.; Sacco, R.L.; Di Tullio, M.R. Patent foramen ovale. *Nat. Rev. Dis. Prim.* **2016**, *2*, 15086. [CrossRef] [PubMed]
2. Meissner, I.; Khandheria, B.K.; Heit, J.A.; Petty, G.W.; Sheps, S.G.; Schwartz, G.L.; Whisnant, J.P.; Wiebers, D.O.; Covalt, J.L.; Petterson, T.M.; et al. Patent foramen ovale: Innocent or guilty? Evidence from a prospective population-based study. *J. Am. Coll. Cardiol.* **2006**, *47*, 440–445. [CrossRef]
3. Koutroulou, I.; Tsivgoulis, G.; Tsalikakis, D.; Karacostas, D.; Grigoriadis, N.; Karapanayiotides, T. Epidemiology of Patent Foramen Ovale in General Population and in Stroke Patients: A Narrative Review. *Front. Neurol.* **2020**, *11*, 281. [CrossRef] [PubMed] [PubMed Central]
4. Hagen, P.T.; Scholz, D.G.; Edwards, W.D. Incidence and size of patent foramen ovale during the first 10 decades of life: An autopsy study of 965 normal hearts. *Mayo Clin. Proc.* **1984**, *59*, 17–20. [CrossRef] [PubMed]
5. Homma, S.; Sacco, R.L. Patent foramen ovale and stroke. *Circulation* **2005**, *112*, 1063–1072. [CrossRef] [PubMed]
6. Serena, J.; Marti-Fàbregas, J.; Santamarina, E.; Rodríguez, J.J.; Perez-Ayuso, M.J.; Masjuan, J.; Segura, T.; Gállego, J.; Dávalos, A. Recurrent stroke and massive right-to-left shunt: Results from the prospective Spanish multicenter (CODICIA) study. *Stroke* **2008**, *39*, 3131–3136. [CrossRef] [PubMed]
7. Alsheikh-Ali, A.A.; Thaler, D.E.; Kent, D.M. Patent foramen ovale in cryptogenic stroke: Incidental or pathogenic? *Stroke* **2009**, *40*, 2349–2355. [CrossRef] [PubMed] [PubMed Central]
8. Katsanos, A.H.; Bhole, R.; Frogoudaki, A.; Giannopoulos, S.; Goyal, N.; Vrettou, A.R.; Ikonomidis, I.; Paraskevaidis, I.; Pappas, K.; Parissis, J.; et al. The value of transesophageal echocardiography for embolic strokes of undetermined source. *Neurology* **2016**, *87*, 988–995. [CrossRef] [PubMed] [PubMed Central]
9. Dagra, A.; Lucke-Wold, B.; McGrath, K.; Mehkri, I.; Mehkri, Y.; Davidson, C.G.; Gilberstadt, N.; Douglas, B.W.; Hoh, B. Central Retinal Artery Occlusion: A Review of Pathophysiological Features and Management. *Stroke Vasc. Interv. Neurol.* **2024**, *4*, e000977. [CrossRef]
10. Schwedt, T.J.; Demaerschalk, B.M.; Dodick, D.W. Patent foramen ovale and migraine: A quantitative systematic review. *Cephalalgia* **2008**, *28*, 531–540. [CrossRef]
11. Collado, F.M.S.; Poulin, M.F.; Murphy, J.J.; Jneid, H.; Kavinsky, C.J. Patent Foramen Ovale Closure for Stroke Prevention and Other Disorders. *J. Am. Heart Assoc.* **2018**, *7*, e007146. [CrossRef] [PubMed]
12. Homma, S.; Di Tullio, M.R.; Sacco, R.L.; Mihalatos, D.; Li Mandri, G.; Mohr, J.P. Characteristics of patent foramen ovale associated with cryptogenic stroke. A biplane transesophageal echocardiographic study. *Stroke* **1994**, *25*, 582–586. [CrossRef] [PubMed]
13. Goel, S.S.; Tuzcu, E.M.; Shishehbor, M.H.; de Oliveira, E.I.; Borek, P.P.; Krasuski, R.A.; Rodriguez, L.L.; Kapadia, S.R. Morphology of the patent foramen ovale in asymptomatic versus symptomatic (stroke or transient ischemic attack) patients. *Am. J. Cardiol.* **2009**, *103*, 124–129. [CrossRef] [PubMed]
14. Clarke, N.R.A.; Timperley, J.; Kelion, A.D.; Banning, A.P. Transthoracic echocardiography using second harmonic imaging with Valsalva manoeuvre for the detection of right to left shunts. *Eur. J. Echocardiogr. J. Work. Gr. Echocardiogr. Eur. Soc. Cardiol.* **2004**, *5*, 176–181. [CrossRef] [PubMed]
15. Daniëls, C.; Weytjens, C.; Cosyns, B.; Schoors, D.; Desutter, J.; Paelinck, B.; Muyldermans, L.; Vancamp, G. Second harmonic transthoracic echocardiography: The new reference screening method for the detection of patent foramen ovale. *Eur. J. Echocardiogr. J. Work. Gr. Echocardiogr. Eur. Soc. Cardiol.* **2004**, *5*, 449–452. [CrossRef] [PubMed]
16. Takaya, Y.; Nakayama, R.; Akagi, T.; Yokohama, F.; Miki, T.; Nakagawa, K.; Toh, N.; Ito, H. Importance of saline contrast transthoracic echocardiography for evaluating large right-to-left shunt in patent foramen ovale associated with cryptogenic stroke. *Int. J. Cardiovasc. Imaging* **2022**, *38*, 515–520. [CrossRef] [PubMed] [PubMed Central]
17. Schneider, B.; Zienkiewicz, T.; Jansen, V.; Hofmann, T.; Noltenius, H.; Meinertz, T. Diagnosis of patent foramen ovale by transesophageal echocardiography and correlation with autopsy findings. *Am. J. Cardiol.* **1996**, *77*, 1202–1209. [CrossRef] [PubMed]
18. Zanette, E.M.; Mancini, G.; De Castro, S.; Solaro, M.; Cartoni, D.; Chiarotti, F. Patent foramen ovale and transcranial Doppler: Comparison of different procedures. *Stroke* **1996**, *27*, 2251–2255. [CrossRef] [PubMed]
19. Job, F.P.; Ringelstein, E.; Grafen, Y.; Flachskampf, F.A.; Doherty, C.; Stockmanns, A.; Hanrath, P. Comparison of transcranial contrast Doppler sonography and transesophageal contrast echocardiography for the detection of patent foramen ovale in young stroke patients. *Am. J. Cardiol.* **1994**, *74*, 381–384. [CrossRef]
20. Klötzsch, C.; Janssen, G.; Berlit, P. Transesophageal echocardiography and contrast-TCD in the detection of a patent foramen ovale: Experiences with 111 patients. *Neurology* **1994**, *44*, 1603–1606. [CrossRef]
21. Mojadidi, M.K.; Roberts, S.C.; Winoker, J.S.; Romero, J.; Goodman-Meza, D.; Gevorgyan, R.; Tobis, J.M. Accuracy of transcranial Doppler for the diagnosis of intracardiac right-to-left shunt: A bivariate meta-analysis of prospective studies. *JACC Cardiovasc. Imaging* **2014**, *7*, 236–250. [CrossRef] [PubMed]
22. Van, H.; Poommipanit, P.; Shalaby, M.; Gevorgyan, R.; Tseng, C.H.; Tobis, J. Sensitivity of transcranial Doppler versus intracardiac echocardiography in the detection of right-to-left shunt. *JACC Cardiovasc. Imaging* **2010**, *3*, 343–348. [CrossRef]
23. Hayreh, S.S. Ocular vascular occlusive disorders: Natural history of visual outcome. *Prog. Retin. Eye Res.* **2014**, *41*, 1–25. [CrossRef] [PubMed]

24. Kvickström, P.; Lindblom, B.; Bergström, G.; Zetterberg, M. Amaurosis fugax: Risk factors and prevalence of significant carotid stenosis. *Clin. Ophthalmol.* **2016**, *10*, 2165–2170. [CrossRef] [PubMed]
25. Sverdlichenko, I.; Donaldson, L.; Margolin, E. Yield of Investigations in Young Patients Presenting With Transient Monocular Vision Loss: A Prospective Study. *Am. J. Ophthalmol.* **2024**, *257*, 137–142. [CrossRef] [PubMed]
26. Clifford, L.; Sievers, R.; Salmon, A.; Newsom, R.S.B. Central retinal artery occlusion: Association with patent foramen ovale. *Eye* **2006**, *20*, 736–738. [CrossRef] [PubMed]
27. Chang, Y.S.; Ho, C.H.; Chu, C.C.; Wang, J.J.; Tseng, S.H.; Jan, R.L. Risk of retinal artery occlusion in patients with diabetes mellitus: A retrospective large-scale cohort study. *PLoS ONE* **2018**, *13*, e0201627. [CrossRef] [PubMed]
28. Fekri, S.; Mahmoudimehr, P.; Jafari Fesharaki, M.; Hosseinjani, E.; Abtahi, S.H.; Nouri, H. Retinal arterial occlusion and patent foramen ovale: A case study-based review. *J. Fr. Ophtalmol.* **2024**, *47*, 104021. [CrossRef] [PubMed]
29. Schneider, M.; Molnar, A.; Angeli, O.; Szabo, D.; Bernath, F.; Hajdu, D.; Gombocz, E.; Mate, B.; Jiling, B.; Nagy, B.V.; et al. Prevalence of Cilioretinal Arteries: A systematic review and a prospective cross-sectional observational study. *Acta Ophthalmol.* **2021**, *99*, e310–e318. [CrossRef]
30. Draper, E.M.; Malloy, K.A. Case Report: Retinal Artery Occlusion Associated with Patent Foramen Ovale. *Clin. Insights Eyecare* **2023**, *1*, 1–9.
31. Jürgens, L.; Yaici, R.; Schnitzler, C.M.; Fleitmann, A.K.; Roth, M.; Schröder, K.; Guthoff, R. Retinal vascular occlusion in pregnancy: Three case reports and a review of the literature. *J. Med. Case Rep.* **2022**, *16*, 167. [CrossRef] [PubMed]
32. Wieder, M.S.; Blace, N.; Szlechter, M.M.; Shulman, E.; Thankenchen, J.; Mbekeani, J.N. Central retinal artery occlusion associated with patent foramen ovale: A case report and literature review. *Arq. Bras. Oftalmol.* **2021**, *84*, 494–498. [CrossRef]
33. Chatziralli, I.P.; Parikakis, E.A.; Mitropoulos, P.G. Undiagnosed patent foramen ovale as a rare cause for branch retinal artery occlusion. *Eur. J. Ophthalmol.* **2015**, *25*, e88–e90. [CrossRef]
34. Biousse, V.; Nahab, F.; Newman, N.J. Management of Acute Retinal Ischemia: Follow the Guidelines! *Ophthalmology* **2018**, *125*, 1597–1607. [CrossRef] [PubMed]
35. Sharma, S.; Sharma, S.M.; Cruess, A.F.; Brown, G.C. Transthoracic echocardiography in young patients with acute retinal arterial obstruction. RECO Study Group. Retinal Emboli of Cardiac Origin Group. *Can. J. Ophthalmol.* **1997**, *32*, 38–41.
36. Inatomi, Y.; Hino, H.; Hashimoto, Y.; Furuyoshi, N.; Misumi, I.; Uchino, M. Transesophageal echocardiography for detection of cardiac diseases in patients with retinal artery occlusion. *Intern. Med.* **2001**, *40*, 475–478. [CrossRef]
37. Tayyab, H.; Aamir, F.B.; Kirmani, S. Central retinal artery occlusion as a result of symptomatic patent foramen ovale. *Rom. J. Ophthalmol.* **2023**, *67*, 69–72. [CrossRef]
38. Ho, I.V.; Spaide, R. Central retinal artery occlusion associated with a patent foramen ovale. *Retina* **2007**, *27*, 259–260. [CrossRef] [PubMed]
39. Gabrielian, A.; Mieler, W.F.; Hariprasad, S.M. Retinal artery occlusion associated with a patent foramen ovale. *Eye* **2010**, *24*, 396–397. [CrossRef]
40. Nakagawa, T.; Hirata, A.; Inoue, N.; Hashimoto, Y.; Tanihara, H. A case of bilateral central retinal artery obstruction with patent foramen ovale. *Acta Ophthalmol. Scand.* **2004**, *82*, 111–112. [CrossRef]
41. Ascuitto, M.; Guertin, D.; Weaver, K. Acute unilateral vision loss from central retinal artery occlusion associated with an atrial septal aneurysm and a patent foramen ovale: A case report. *Fam. Pract.* **2021**, *38*, 834–836. [CrossRef] [PubMed]
42. Shoeibi, N.; Poorzand, H.; Abrishami, M. Branch retinal artery occlusion in a patient with patent foramen ovale. *J. Ophthalmic Vis. Res.* **2013**, *8*, 58–61. [PubMed]
43. Wisotsky, B.J.; Engel, H.M. Transesophageal echocardiography in the diagnosis of branch retinal artery obstruction. *Am. J. Ophthalmol.* **1993**, *115*, 653–656. [CrossRef] [PubMed]
44. Liu, X.; Calvert, P.A.; Arif, S.; Keane, P.A.; Denniston, A.K. Patent foramen ovale presenting as visual loss. *JRSM Open* **2016**, *8*, 2054270416669302. [CrossRef] [PubMed]
45. Grudzińska, E.; Nowacka, B.; Bania, B.; Lubiński, W.; Zaborski, D.; Modrzejewska, M. Patent foramen ovale as a cause of acute vision loss. *Int. J. Ophthalmol.* **2021**, *14*, 1125–1126. [CrossRef] [PubMed]
46. Sheth, H.G.; Laverde-Konig, T.; Raina, J. Undiagnosed patent foramen ovale presenting as retinal artery occlusion-an emerging association. *J. Ophthalmol.* **2009**, *2009*, 248269. [CrossRef] [PubMed]
47. Sabanis, N.; Zagkotsis, G.; Krikos, V.D.; Paschou, E.; Tapanlis, A. Central Retinal Artery Occlusion Secondary to Patent Foramen Ovale: The Unexpected Journey of a Paradoxical Embolus. *Cureus* **2020**, *12*, e9496. [CrossRef] [PubMed]
48. Kramer, M.; Goldenberg-Cohen, N.; Shapira, Y.; Axer-Siegel, R.; Shmuely, H.; Adler, Y.; Weinberger, D.; Sagie, A. Role of transesophageal echocardiography in the evaluation of patients with retinal artery occlusion. *Ophthalmology* **2001**, *108*, 1461–1464. [CrossRef]
49. Zhu, L.; Xu, N.; Li, Y. Monocular central retinal artery occlusion caused by bilateral internal carotid artery hypoplasia complicated with patent foramen ovale: A case report and review of literature. *Eur. J. Med. Res.* **2021**, *26*, 55. [CrossRef]
50. Si, M.; Wang, H. Retinal artery occlusion after facial filler injection in a patient with patent foramen ovale: A case report and literature review. *J. Int. Med. Res.* **2023**, *51*, 3000605231194514. [CrossRef]
51. Pipolo, G.R.; Neto, C.A.M.; Ishida, P.; Pereira, R.; Torres, R.; Barreto, G.; Moreira, C.A.M., Jr. OCT Angiography Use in Pregnancy: Branch Retinal Artery Occlusion Associated with Patent Foramen Ovale—A Case Report and Multimodal Analysis. *Case Rep. Ophthalmol.* **2023**, *14*, 29–33. [CrossRef] [PubMed]

52. Kapoor, K.M.; Kapoor, P.; Heydenrych, I.; Bertossi, D. Vision Loss Associated with Hyaluronic Acid Fillers: A Systematic Review of Literature. *Aesthetic Plast. Surg.* **2020**, *44*, 929–944. [CrossRef] [PubMed]
53. Kanwar, S.M.; Noheria, A.; DeSimone, C.V.; Rabinstein, A.A.; Asirvatham, S.J. Coincidental Impact of Transcatheter Patent Foramen Ovale Closure on Migraine with and without Aura—A Comprehensive Meta-Analysis. *Clin. trials Regul. Sci. Cardiol.* **2016**, *15*, 7–13. [CrossRef]
54. Lipton, R.B.; Stewart, W.F.; Diamond, S.; Diamond, M.L.; Reed, M. Prevalence and burden of migraine in the United States: Data from the American Migraine Study II. *Headache* **2001**, *41*, 646–657. [CrossRef] [PubMed]
55. Del Sette, M.; Dinia, L.; Bonzano, L.; Roccatagliata, L.; Finocchi, C.; Parodi, R.; Sivori, G.; Gandolfo, C. White matter lesions in migraine and right-to-left shunt: A conventional and diffusion MRI study. *Cephalalgia* **2008**, *28*, 376–382. [CrossRef] [PubMed]
56. Liu, K.; Wang, B.Z.; Hao, Y.; Song, S.; Pan, M. The Correlation Between Migraine and Patent Foramen Ovale. *Front. Neurol.* **2020**, *11*, 543485. [CrossRef] [PubMed]
57. Wilmshurst, P.T. Migraine with aura and persistent foramen ovale. *Eye* **2018**, *32*, 184–188. [CrossRef] [PubMed]
58. Dowson, A.; Mullen, M.J.; Peatfield, R.; Muir, K.; Khan, A.A.; Wells, C.; Lipscombe, S.L.; Rees, T.; De Giovanni, J.V.; Morrison, W.L.; et al. Migraine Intervention With STARFlex Technology (MIST) trial: A prospective, multicenter, double-blind, sham-controlled trial to evaluate the effectiveness of patent foramen ovale closure with STARFlex septal repair implant to resolve refractory migraine. *Circulation* **2008**, *117*, 1397–1404. [CrossRef]
59. Mattle, H.P.; Evers, S.; Hildick-Smith, D.; Becker, W.J.; Baumgartner, H.; Chataway, J.; Gawel, M.; Göbel, H.; Heinze, A.; Horlick, E.; et al. Percutaneous closure of patent foramen ovale in migraine with aura, a randomized controlled trial. *Eur. Heart J.* **2016**, *37*, 2029–2036. [CrossRef]
60. Tobis, J.M.; Charles, A.; Silberstein, S.D.; Sorensen, S.; Maini, B.; Horwitz, P.A.; Gurley, J.C. Percutaneous Closure of Patent Foramen Ovale in Patients With Migraine: The PREMIUM Trial. *J. Am. Coll. Cardiol.* **2017**, *70*, 2766–2774. [CrossRef]
61. Giardini, A.; Donti, A.; Formigari, R.; Salomone, L.; Palareti, G.; Guidetti, D.; Picchio, F.M. Long-term efficacy of transcatheter patent foramen ovale closure on migraine headache with aura and recurrent stroke. *Catheter. Cardiovasc. Interv. Off. J. Soc. Card. Angiogr. Interv.* **2006**, *67*, 625–629. [CrossRef]
62. Yan, S.; Wang, N.; Liu, H.; Wei, Y.; Liu, X.; Wang, L.; Huang, Y.; Lin, M. Patent Foramen Ovale Closure for Migraineurs with Massive Right-to-Left Shunt and White Matter Lesions: An Exploration on Curative Effects. *Altern. Ther. Health Med.* **2024**, AT9388, *ahead of print*. [PubMed]
63. Elbadawi, A.; Barssoum, K.; Abuzaid, A.S.; Rezq, A.; Biniwale, N.; Alotaki, E.; Mohamed, A.H.; Vuyyala, S.; O Ogunbayo, G.; Saad, M. Meta-analysis of randomized trials on percutaneous patent foramen ovale closure for prevention of migraine. *Acta Cardiol.* **2019**, *74*, 124–129. [CrossRef] [PubMed]
64. Shi, Y.-J.; Lv, J.; Han, X.-T.; Luo, G.-G. Migraine and percutaneous patent foramen ovale closure: A systematic review and meta-analysis. *BMC Cardiovasc. Disord.* **2017**, *17*, 203. [CrossRef] [PubMed]
65. Zhang, Y.; Wang, H.; Liu, L. Patent Foramen Ovale Closure for Treating Migraine: A Meta-Analysis. *J. Interv. Cardiol.* **2022**, *2022*, 6456272. [CrossRef]
66. Zhang, Q.-Q.; Lu, J.-J.; Yan, M.-Y.; Hu, X.-W.; Qin, Y.-R.; Wang, D.-P.; Jiang, J.-H.; Fang, Q.; Zhao, H.-R. The Efficacy of Percutaneous Patent Foramen Ovale Closure on Migraine: A Meta-Analysis of Randomized Controlled Trials and Observational Studies. *Biomed. Res. Int.* **2021**, *2021*, 6643266. [CrossRef]
67. Arunagiri, G.; Santhi, S. Migraine: An ophthalmologist's perspective. *Curr. Opin. Ophthalmol.* **2003**, *14*, 344–352. [CrossRef] [PubMed]
68. Ciuffreda, K.J.; Kapoor, N.; Rutner, D.; Suchoff, I.B.; Han, M.E.; Craig, S. Occurrence of oculomotor dysfunctions in acquired brain injury: A retrospective analysis. *Optometry* **2007**, *78*, 155–161. [CrossRef] [PubMed]
69. Xie, D.N.; Sun, Q. Bilateral ptosis caused by patent foramen ovale-associated stroke. *QJM An. Int. J. Med.* **2022**, *115*, 853–854. [CrossRef]
70. Mazurkiewicz-Bełdzińska, M.; Szmuda, M.; Zawadzka, M. Internuclear opthalmoplegia as a symptom of ischemic stroke in a girl with patent foramen ovale. *Pediatr. Neurol.* **2015**, *52*, 466–467. [CrossRef]
71. Zhuang, S.; Xie, W.; Mao, C. Bilateral facial colliculus syndrome caused by pontine tegmentum infarction: A case report. *BMC Neurol.* **2021**, *21*, 492. [CrossRef] [PubMed]
72. Takahashi, C. A Case of Progressive Stroke on Posterior Circulation with Transient Bilateral Oculomotor Palsy. *Case Rep. Neurol. Med.* **2018**, *2018*, 1579426. [CrossRef] [PubMed]
73. Khan, A.O. Pupil-sparing complete third nerve palsy from cryptogenic midbrain stroke in an otherwise-healthy young adult with patent foramen ovale. *Middle East. Afr. J. Ophthalmol.* **2012**, *19*, 237–239. [CrossRef] [PubMed]
74. Durand, M.L. Endophthalmitis. *Clin. Microbiol. Infect. Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis.* **2013**, *19*, 227–234. [CrossRef] [PubMed]
75. Sadiq, M.A.; Hassan, M.; Agarwal, A.; Sarwar, S.; Toufeeq, S.; Soliman, M.K.; Hanout, M.; Sepah, Y.J.; Do, D.V.; Nguyen, Q.D. Endogenous endophthalmitis: Diagnosis, management, and prognosis. *J. Ophthalmic Inflamm. Infect.* **2015**, *5*, 32. [CrossRef] [PubMed]
76. Rodrigues, A.C.; Ogawa, A.; Mota, J.; Carbone, A.; Arruda, A.L.; Furtado, M.; Pinheiro, J.; Andrade, J.L. Endophthalmitis as a first manifestation of right-sided endocarditis in a patient with patent foramen ovale. *J. Echocardiogr.* **2012**, *10*, 67–68. [CrossRef] [PubMed]

77. Nii, T.; Yoshikawa, H.; Okabe, T.; Tachibana, I. Septic pulmonary and systemic embolism in tricuspid endocarditis. *BMJ Case Rep.* **2014**, *2014*, 206569. [CrossRef] [PubMed]
78. Flaxel, C.J.; Adelman, R.A.; Bailey, S.T.; Fawzi, A.; Lim, J.I.; Vemulakonda, G.A.; Ying, G.-S. Retinal and Ophthalmic Artery Occlusions Preferred Practice Pattern®. *Ophthalmology* **2020**, *127*, P259–P287. [CrossRef] [PubMed]
79. Endophthalmitis Study Group, European Society of Cataract & Refractive Surgeons. Prophylaxis of postoperative endophthalmitis following cataract surgery: Results of the ESCRS multicenter study and identification of risk factors. *J. Cataract. Refract. Surg.* **2007**, *33*, 978–988. [CrossRef] [PubMed]
80. Vukadinović, D.; Schirmer, S.H.; Vukadinović, A.N.; Ukena, C.; Scheller, B.; Mahfoud, F.; Böhm, M. Interventional closure vs. medical therapy of patent foramen ovale for secondary prevention of stroke: Updated meta-analysis. *Clin. Res. Cardiol.* **2019**, *108*, 157–166. [CrossRef]
81. Turc, G.; Calvet, D.; Guérin, P.; Sroussi, M.; Chatellier, G.; Mas, J.L. Closure, Anticoagulation, or Antiplatelet Therapy for Cryptogenic Stroke With Patent Foramen Ovale: Systematic Review of Randomized Trials, Sequential Meta-Analysis, and New Insights from the CLOSE Study. *J. Am. Heart Assoc.* **2018**, *7*, e008356. [CrossRef] [PubMed]
82. Liu, Y.; Wu, Y.; Xiong, L. Surgical vs. drug therapy in patients with patent foramen ovale and cryptogenic stroke. *Herz* **2021**, *46*, 250–254. [CrossRef] [PubMed]
83. Rim, T.H.; Lee, C.J.; Tham, Y.C.; Cheung, N.; Yu, M.; Lee, G.; Kim, Y.; Ting, D.S.W.; Chong, C.C.Y.; Choi, Y.S.; et al. Deep-learning-based cardiovascular risk stratification using coronary artery calcium scores predicted from retinal photographs. *Lancet Digit. Health* **2021**, *3*, e306–e316. [CrossRef] [PubMed]
84. Wagner, S.K.; Fu, D.J.; Faes, L.; Liu, X.; Huemer, J.; Khalid, H.; Ferraz, D.; Korot, E.; Kelly, C.; Balaskas, K.; et al. Insights into Systemic Disease through Retinal Imaging-Based Oculomics. *Transl. Vis. Sci. Technol.* **2020**, *9*, 6. Erratum in *Transl. Vis. Sci. Technol.* **2021**, *10*, 13. https://doi.org/10.1167/tvst.10.8.13. [CrossRef] [PubMed]

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Review

The Role of Oral Supplementation for the Management of Age-Related Macular Degeneration: A Narrative Review

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Abstract: The majority of neurodegenerative eye disorders occur with aging and significantly impair quality of life. Age-related macular degeneration (AMD) is the third most common cause of visual impairment and blindness worldwide. One of the most important elements in the pathophysiology of neurodegenerative eye disease is certainly oxidative stress, with neuroinflammation and ocular ischemia which may also be significant factors. Antioxidants, either by food or oral supplementation, may be able to mitigate the deleterious effects of reactive oxygen species that build as a result of oxidative stress, ischemia, and inflammation. Over the past few decades, a number of research works examining the potential adjuvant impact of antioxidants in AMD have been published. In fact, there is not only more and more interest in already known molecules but also in new molecules that can help clinicians in the management of this complex multifactorial disease, such as astaxanthin and melatonin. However, while some studies showed encouraging outcomes, others were conflicting. In addition, more and more attention is also being paid to nutrition, considered a pivotal key point, especially to prevent AMD. For this reason, the purpose of this review is to analyze the main antioxidant molecules currently used as oral supplements for AMD treatment, as well as the role of diet and food intake in this ocular disease, to better understand how all these factors can improve the clinical management of AMD patients.

Keywords: age-related macular degeneration; AMD; diet; food intake; oral supplementation

1. Introduction

Age-related macular degeneration (AMD) is the third most common cause of severe irreversible vision loss worldwide and it is considered the leading cause of central blindness in industrialized countries, especially in people over 60 years of age [1]. In fact, prevalence data suggest that AMD affects around 200 million people today and it is expected to rise further to almost 300 million by 2040 [1].

AMD is a multifactorial disorder with multiple genetic and environmental risk factors, including genetic predisposition, older age, smoking, obesity, low dietary intake of several vitamins and minerals (A, C, E, and zinc), low dietary intake of lutein and omega-3 fatty acids, and unhealthy lifestyle related to cardiovascular risk factors [2,3].

In 2001, the Age-Related Eye Disease Study (AREDS) [4] first showed that patients with advanced AMD, or those with at least intermediate AMD (defined as bilateral large drusen with or without pigment changes) [5], could benefit from taking oral antioxidant vitamin and mineral supplements [4].

Indeed, one pivotal element in the pathophysiology of neurodegenerative eye disease such as AMD is certainly oxidative stress [6] (Figure 1).

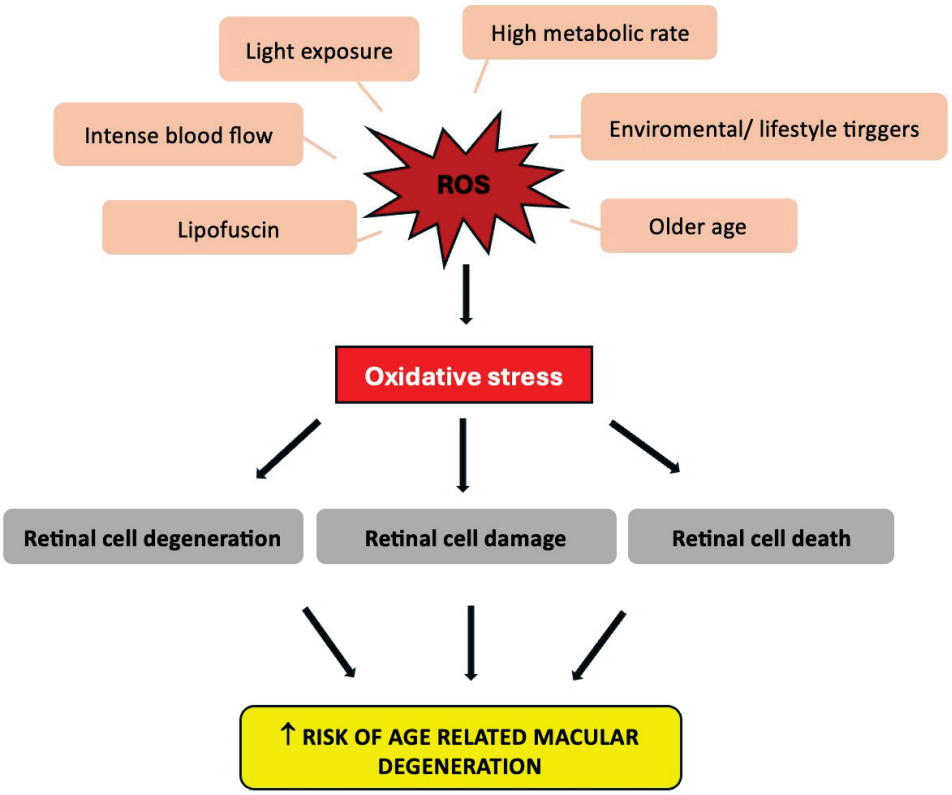

Figure 1. Factors predisposing the formation of reactive oxygen species (ROS), responsible for oxidative stress which can lead to an increased risk of age-related macular degeneration.

In response to oxidative stress, which can be induced by molecules like lipopolysaccharides, exosomes derived from retinal pigment epithelial cells (RPE) can induce inflammation in the retina and thus contribute to AMD pathogenesis. Among the key genes involved in oxidative stress, interleukin-1 beta (IL-1β), interleukin-6 (IL-6), Nuclear Factor Kappa B (NF-κB), and tumor necrosis factor alpha (TNF-α) are susceptible to upregulation [7], thus triggering an inflammatory cascade that contributes to disease progression [8].

Moreover, neuroinflammation and ocular ischemia also have a significant impact on the disease. Antioxidants, either by food or oral supplementation, may be able to mitigate the deleterious effects of reactive oxygen species (ROS) that build because of oxidative stress, ischemia, and inflammation by reducing the expression of the genes involved in retinal inflammation [6].

Over the past few decades, several studies examining the potential impact of antioxidants in AMD have been published [9,10], showing their potential use in AMD management and also as adjuvants to the therapeutic action of intravitreal anti-vascular endothelial growth factor (VEGF) drugs.

For this reason, the purpose of this narrative review was to explore a wide range of active substances, including not only vitamins and minerals as antioxidants, but also other substances such as saffron, curcumin, and melatonin, which are currently used as oral supplements for AMD. Our work differs from previous studies by including a broader range of these compounds [11]. Additionally, given the growing interest in understanding the impact of dietary factors on AMD progression, we aimed to integrate recent evidence on the role of diet and food intake in the management of this disease.

2. Main Oral Supplements for AMD Management

2.1. Lutein and Zeaxanthin

Lutein and zeaxanthin are pigments produced by plants and belong to the carotenoid family of xanthophylls. They are two fat-soluble antioxidants with very similar structures, differing in the location of one double bond in one of the hydroxyl groups. Lutein and zeaxanthin are only biosynthesized by plants, so these substances must be provided to the body through diet. Major sources of these carotenoids include broccoli, kale, spinach, corn, peas, and egg yolks. The recommended daily intake of lutein is about 10.0 mg, while for zeaxanthin, it is 2 mg [12–14]. Lutein and zeaxanthin are the only dietary carotenoids that accumulate in the retina, especially in the macula and in the lens of the human eye, and are called macular pigments, responsible for fine detail vision [12,15].

Given their accumulation in the retina, researchers have investigated the role of lutein and zeaxanthin in eye health. Their functions include enhancing visual function [16,17], acting as antioxidants to scavenge free radicals and protect the macula from oxidative damage [18,19], and filtering blue light [19,20]. Lutein and zeaxanthin are the most potent antioxidants for the prevention or risk reduction of AMD and various eye-related conditions [21]. Evidence suggests that these xanthophylls may protect the retina from damage and could potentially prevent or slow down the progression of AMD.

Numerous studies have explored the impact of lutein and zeaxanthin supplementation (both through whole foods and supplements) and their association with AMD. A multicenter case–control study conducted across five ophthalmological centers in the United States was the first epidemiological study to demonstrate a direct relationship between lutein intake and the risk of AMD [22]. Increasing the consumption of foods rich in specific carotenoids, particularly dark green, leafy vegetables, may help reduce the risk of developing advanced or exudative AMD [22].

The evidence supporting the protective effects of lutein and zeaxanthin intake against AMD development comes from the Age-Related Eye Disease Study 2 (AREDS2) published in 2013, an important clinical trial conducted in the United States [23]. The original Age-Related Eye Disease Study (AREDS1) was published in 2001 and was one of the first significant clinical trials to assess the association between nutrient intake and the progression of AMD [4]. In this original study, it was demonstrated that a combination of nutrient supplements (500 mg vitamin C, 400 IU vitamin E, 15 mg β-carotene, 80 mg zinc, and 2 mg cupric oxide) taken daily reduces the risk of developing advanced macular degeneration by 25% [4]. However, subsequent findings linking β-carotene to an increased risk of lung cancer among smokers led to revisions in the formulation [24]. Later, AREDS2 included lutein (10 mg) and zeaxanthin (2 mg) along with omega-3 long-chain polyunsaturated fatty acids, eliminating beta carotene and reducing zinc doses in the formulation [23]. While this modified formula did not further reduce the risk of late AMD progression compared to the previous one, the removal of β-carotene and the addition of lutein and zeaxanthin provided similar protective effects without introducing additional risks for smokers [23].

These studies have provided valuable clinical guidance on dietary supplementation for AMD patients [4,23]. Evidence also indicates that higher intake of lutein and zeaxanthin may offer significant protection against AMD, especially in individuals with a high genetic risk based on two major AMD genes [25]. A meta-analysis [21] involving seven controlled trials using lutein and zeaxanthin supplements [16,26–33] assessed the impact of this supplementation on visual performance in individuals with established AMD. The results of the meta-analysis suggest that lutein and zeaxanthin supplementation represents a safe and effective strategy for enhancing the visual performance and contrast sensitivity of AMD patients in a dose–response manner [26].

Determining effective doses of lutein and zeaxanthin is challenging due to a lack of long-term studies. However, current evidence suggests that higher dietary intake of these compounds may protect against AMD. A diverse diet rich in leafy greens and other foods can help achieve optimal levels of lutein and zeaxanthin, thus supporting eye health.

Further research is needed to improve our understanding of lutein and zeaxanthin and establish recommended targets for these eye-protective carotenoids.

2.2. Astaxanthin

Astaxanthin (3,3′-dihydroxy-β, β-carotene4,4′-dione), is a carotenoid that belongs to the family of oxygenated derivatives of carotenoids (xanthophylls) present in nature, mainly in marine environments, where it occurs as a red pigment, contributing to the red color of shrimp, lobsters, and shrimp pulp. Astaxanthin is biosynthesized by phytoplankton and microalgae, accumulating in various aquatic species that represent the main dietary sources of this carotenoid. It is a bioactive compound with promising structural and functional characteristics in the prevention of numerous human diseases as well as in maintaining good health [34,35].

Unlike most antioxidants, astaxanthin spans across the double layer of the membrane, providing protection against oxidative stress by neutralizing ROS and other free radicals both in the polar (hydrophilic) and nonpolar (hydrophobic) boundary zones of the cell membrane [36]. Astaxanthin has been shown to have antioxidant activity approximately ten times higher than that of zeaxanthin and lutein [37], as well as other carotenoids such as α-carotene, lycopene, and β-carotene [38]. Furthermore, it has been observed that astaxanthin can suppress the activation of NF-κB inflammatory gene expression induced by hydrogen peroxide by inhibiting the intracellular ROS accumulation [39]. Indeed, it has demonstrated multiple beneficial effects, including anti-inflammatory, antioxidant, anti-diabetic, and anti-cancer activities in addition to protective actions for the skin and the nervous and cardiovascular systems [36,37,40].

Several recent clinical trials emphasize the potential role of astaxanthin in enhancing eye health, as suggested by the significant improvement observed in the outcomes of various ocular conditions such as age-related macular degeneration, diabetic retinopathy, glaucoma, and cataracts [37].

In a randomized controlled trial, Parisi et al. evaluated the effects of short-term oral supplementation with carotenoids, including astaxanthin, on retinal function in non-advanced AMD patients [41]. Twenty-seven participants were randomly divided into two groups. One group received daily supplementation containing vitamin E (30 mg), vitamin C (180 mg), zinc (22.5 mg), copper (1 mg), lutein (10 mg), zeaxanthin (1 mg), and astaxanthin (4 mg) for 12 months, while the other group received no supplementation. The supplemented group showed improved central retinal function compared to the placebo group, highlighting the potential benefits of carotenoid supplementation in AMD management [41]. In another multicenter, prospective open-label randomized study, 145 patients were randomly divided into two treatment groups [27]. One group received a supplementation containing lutein (10 mg), zeaxanthin (1 mg), astaxanthin (4 mg), and antioxidants/vitamins, while the other group received no dietary supplementation for a duration of two years. Patients treated with lutein/zeaxanthin, astaxanthin, and other nutrients were more inclined to report clinically meaningful stabilization or improvements in visual acuity, contrast sensitivity, and vision-related functions over 24 months compared to untreated subjects [27].

Astaxanthin, due to its potent antioxidant activity, can influence choroidal neovascularization (CNV), another factor contributing to AMD. In fact, CNV is associated with oxidative stress and chronic inflammation in ocular tissues, caused by the overexpression of VEGF. Astaxanthin has demonstrated its ability to suppress CNV in murine experimental models, reducing the formation of abnormal new blood vessels in the retina [42]. These positive effects are supported by molecular mechanisms that regulate the production of inflammatory mediators such as ICAM-1, MCP-1, VEGF, IL-6, and VEGF receptors [42].

In conclusion, astaxanthin offers promising treatment prospects for combating ocular diseases and supporting eye health. However, to define optimal dosages and formulations, improving bioavailability and obtaining more data from clinical studies are essential, despite its broad safety profile and potential efficacy in various ocular conditions [37,43].

2.3. Vitamins and Minerals

High concentrations of certain antioxidant vitamins and minerals in the retina, coupled with the high levels of the carotenoids in the macula, suggest the hypothesis that micronutrient supplementation might protect against AMD. Vitamins A, C, and E are recognized as the most effective for reducing the risk of macular degeneration [4]. Vitamin A is essential for RPE cells of the human retina, while vitamins C and E are known to act as antioxidants. In addition to these vitamins, minerals such as zinc and selenium have also been associated with eye diseases.

AREDS is the first large multicenter randomized, placebo-controlled study initiated by the United States National Eye Institute, designed to investigate the effect of high-dose supplementation with vitamin C and E, beta-carotene, and zinc, either individually or in combination, on the progression of AMD and cataracts [4]. This study enrolled 3640 patients aged between 55 and 80 years diagnosed with AMD. The AREDS1 study showed a statistically significant benefit in consuming the AREDS formula, with a 25% reduced risk of AMD progression over 5 years; although vision loss continued, it occurred at a slower rate with a reduced risk of developing neovascularization [4]. However, the study has several limitations. Firstly, it is unclear to what extent and at what exact dosage vitamin C, vitamin E, and beta-carotene are beneficial for AMD patients because they were administered only in combination and at a single dosage. Additionally, concerns have been raised about the long-term safety of supplementation with dosages that clearly exceed the recommended daily intake [44]. To address some of the original AREDS limitations, the United States National Eye Institute sponsored a second large-scale randomized clinical trial. This study, known as AREDS2, aimed to investigate if additional nutritional supplements beyond AREDS1 supplements could further reduce the risk of progression to advanced AMD [23].

Although AREDS1 and AREDS2 are the most comprehensive studies on vitamin supplementation's impact on AMD progression, smaller-scale studies have also been carried out. However, research findings on the intake of these vitamins and minerals and their association with AMD risk remain conflicting.

2.3.1. β-Carotene and Vitamin A

Beta-carotene is a carotenoid and it is the precursor of vitamin A. Both compounds are liposoluble and excellent antioxidants, capable of counteracting the onset of free radicals [45].

In the AREDS1 study, the supplement formulation included beta-carotene at a dose of 15 mg, based on the structural similarities between the components of the macular pigment and beta-carotene [4]. Therefore, beta-carotene was long part of the recommendation for supplementation in AMD patients. However, it is now established that high doses of β-carotene (30 mg per day) increase the risk of lung cancer in smokers [24]. Although the mechanism behind this harmful effect has not been fully elucidated, side effects do not occur in non-smokers or when the carotenoid is administered at lower doses [46]. Based on these data, it was recommended to omit beta-carotene supplementation in smokers with AMD. Therefore, in the formulation of the AREDS2 study, beta-carotene was replaced with lutein/zeaxanthin [23].

The protective effect of vitamin A against AMD is demonstrated by epidemiological data from the National Health and Nutrition Examination Survey (NHANES I), which showed that those who consumed higher amounts of fruits and vegetables rich in vitamin A had a reduced risk of any stage of AMD [47]. A prospective population-based cohort study has demonstrated that the sustained consumption of fruits and vegetables containing provitamin A carotenoids can further decrease the risk of AMD [48].

However, other studies have not found a significant association between increased dietary vitamin A intake and reduced AMD risk [22,49]. This conflicting evidence highlights the need for additional studies to clarify the relationship between vitamin A and AMD.

2.3.2. Vitamin C

Vitamin C, chemically known as ascorbic acid, is synthesized by plants and certain mammals. However, humans have lost this ability over evolution making it necessary to obtain vitamin C from dietary sources. Therefore, vitamin C is an essential micronutrient for humans, with the recommended daily intake ranging from 75 mg for adult females to 90 mg for adult males per day [50]. Fruits and vegetables are the primary sources of vitamin C in the diet. Vitamin C plays a crucial role as a powerful antioxidant, contributing positively to redox oxidative pathways, inflammation, maintaining endothelial integrity, and regulating lipoprotein metabolism.

Vitamin C is plentiful in the retina, including in premature infants, and its potential protective role in age-related retinal diseases is supported by animal studies. Studies in the mid-1980s showed that ascorbic acid, specifically L-ascorbate, can reduce light-induced damage in rat retinas, likely due to its antioxidant properties [51]. Another study that supports the beneficial effect of Vitamin C is conducted by SanGiovanni et al. that found a reduced risk of neovascular AMD in individuals with a high intake of β-carotene, vitamin C, and vitamin E [52]. However, conflicting results were reported by Evans and Lawrenson, who did not find a significant association between vitamin C and the primary prevention of AMD [11]. This was consistent with other studies that also failed to show a clear link between dietary vitamin C intake and reduced AMD risk, as reported by Delcourt et al. [49] and Seddon et al. [22]. The Eye Diseases Control Study noted a significant correlation between an increased intake of green vegetables and a markedly reduced risk of AMD [53]. However, they found that total vitamin C consumption did not show a significant association with reduced AMD risk [22]. Additionally, the study observed that lower plasma levels of vitamin C were linked to a higher risk of AMD, whereas higher plasma concentrations did not offer protection [22]. Furthermore, a 10-year follow-up study did not find significant effects of vitamin C supplementation on AMD risk [54].

Overall, while some studies suggest a potential protective role for vitamin C against AMD, the evidence remains inconclusive and further research on a larger scale is necessary to clarify this relationship.

2.3.3. Vitamin E

Vitamin E is a fat-soluble vitamin and comprises four chemical compounds, among which α-tocopherol is considered the most biologically active and an effective scavenger of free radicals [55].

The recommended daily dose of 15 mg for adult males is based on α-tocopherol [50]. Photoreceptors and RPE cells, which have a high concentration of vitamin E, respond to increased oxidative stress by increasing their levels of this vitamin [56]. Some evidence supports the idea that vitamin E might offer benefits to those with AMD. Several studies have indicated a correlation between low serum levels of tocopherol and AMD [57,58], suggesting that elevated plasma carotenoid levels may offer protective effects [59].

An interventional study evaluated the effect of vitamin E on 1193 subjects, administering 500 IU of vitamin E (equivalent to 335 mg/day of α-tocopherol) or placebo [60]. After 4 years, no significant benefit was found in vitamin E supplementation for AMD prevention or risk reduction [60].

Most randomized prospective studies evaluating the effect of vitamin E on AMD have administered vitamin E in combination with other antioxidants, not allowing them to establish the actual role of vitamin E in protecting against AMD. In fact, while high blood levels of vitamin E may be considered protective, evidence regarding the influence of vitamin E alone on the incidence or progression of AMD is currently limited.

2.3.4. Zinc

Zinc is an essential trace element, and it is the second most abundant transition metal in the human body, following iron [61]. The human body contains approximately 2 g of this element, with high concentrations in bones, skeletal muscles, liver, and eyes. The eye

exhibits a relatively high concentration of zinc compared to other organs and tissues, with the peak concentration reaching around 300 μg/g of dry tissue in the RPE [62]. Zinc is not stored in the human body; thus, daily diet is the only way for humans to acquire zinc. It occurs naturally in the form of sulfides or oxides and in food, it is most abundant in meat, saltwater fish, sunflower and pumpkin seeds, bran, wheat, egg yolks, onion, garlic, and tea [63]. The recommended daily intake of zinc for adults ranges from 12 to 15 mg/day [50]. Zinc is generally considered safe, particularly when taken orally, although long-term use, even at low doses, may lead to anemia. There is evidence suggesting that zinc can affect the utilization of other nutrients, particularly copper [64].

Zinc acts as a cofactor for hundreds of enzymes across all enzymatic classes and thus participates in a broad range of metabolic functions [65]. Zinc is recognized as important in the pathophysiology of several groups of diseases, including neurodegenerative diseases [62]. Growing evidence suggests that zinc, due to its antioxidant properties, protects cells from the damaging effects of oxidative stress, presumed to be a causative factor in the development of various age-related retinal disorders [66,67].

The first study assessing the effects of oral zinc administration in AMD patients was conducted in 1988 by Newsome et al. [68]. This was a randomized, 2-year, placebo-controlled clinical trial involving 151 subjects with drusen or macular degeneration, finding a statistically significant reduction in visual acuity loss in the group treated with zinc [68]. The study recommended a more definitive trial before making a general recommendation for zinc supplementation in individuals at risk of vision loss from advanced AMD [68]. Contrary to this finding, two epidemiological studies did not confirm this outcome, where zinc supplementation had no short-term effect on the progression of AMD [69] and was not associated with a reduced risk of AMD [70]. After these, the large randomized, placebo-controlled AREDS/AREDS2 studies were published [4,23]. These studies evaluated supplementation with relatively high doses of some vitamins with or without some minerals, including zinc administered as zinc oxide at 80 mg and copper at 2 mg per day. Copper was included in the AREDS formulations along with zinc to prevent copper deficiency anemia, which can occur due to metabolic competition with zinc during supplementation. These studies suggested that zinc supplementation in combination with the AREDS formula and other antioxidants could suppress retinal degeneration [71].

In conclusion, zinc supplementation has shown potential benefits for AMD patients in certain studies, with some indicating a slowdown in progression. However, there is no universal consensus on its efficacy, as other studies either found no effect or, in rare cases, even suggested an adverse effect [62].

2.3.5. Selenium

Selenium is an essential trace element in human body, with recommended daily levels set at 55 μg for both adult women and men [50]. Known for its antioxidant properties, recent studies have explored whether selenium can mitigate the risk of AMD, especially through its role in selenium-dependent glutathione peroxidase, which protects cellular lipids from oxidative damage [72]. However, current evidence is inconclusive and does not clearly confirm that selenium can effectively protect the macula from AMD [53]. The role of selenium in protecting against AMD is still unclear due to a lack of case–control studies. Further research is needed to determine if selenium can effectively prevent AMD and associated eye complications.

2.4. Omega-3 Fatty Acids

Omega-3 fatty acids are polyunsaturated fatty acids (PUFAs) defined as essential fats because humans lack the enzymatic mechanism necessary to synthesize them [73]. Their primary sources include fatty fish such as mackerel, salmon, trout, and tuna, which are particularly rich in omega-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [74]. Omega-3 PUFA supplements, such as fish oil, are popular for their recognized anti-inflammatory properties [75]. EPA and DHA can modulate the immune response

by reducing the production of pro-inflammatory cytokines (TNF-α, IL-1, IL-6, LTB4) and leukocyte activation, and by increasing anti-inflammatory derivatives like PGD2 [76,77]. Their anti-inflammatory mechanisms include altering the composition of phospholipid fatty acids in cell membranes, disrupting lipid rafts, and inhibiting the pro-inflammatory nuclear transcription factor κB activation, thereby reducing inflammatory gene expression and activating anti-inflammatory responses [78]. Although generally safe and well-tolerated, omega-3 fatty acid supplements may exacerbate bleeding and anticoagulation in individuals using anticoagulants [79].

In recent years, there has been growing interest in the potential protective role of omega-3 PUFAs against various retinal diseases linked to inflammation, ischemia, light exposure, oxidative stress, and aging [80]. DHA is a major structural component of the retina, and EPA may play a role as a precursor to signaling molecules with a potential role to influence retinal function [76]. PUFAs have also shown anti-inflammatory and antioxidant properties, anti-angiogenic, anti-vasoproliferative, and neuroprotective effects [76].

Studies on animal models of macular degeneration have also demonstrated encouraging outcomes with PUFA supplementation [77–82]. Moreover, they reduce pathological angiogenesis across cellular and animal models by influencing numerous angiogenic factors like platelet-derived growth factor (PDGF) and VEGF [80,83]. The AREDS2 trial included, in addition to lutein and zeaxanthin, omega-3 long-chain PUFAs (DHA 350 mg + EPA 650 mg) in the oral formulation for treating the progression to advanced AMD [23]. Overall, there was no additional benefit observed from the addition of these components to the formulation. Although the addition of omega-3 to the AREDS formulation did not prove to be beneficial, it is believed that higher doses of EPA and DHA may have a desirable effect [84]. Additionally, unsaturated fats aid in the absorption of lutein and zeaxanthin [12].

In an observational study conducted by Georgiou and Prokopiou, patients with dry AMD were supplemented with EPA and DHA for up to 6 months at doses ranging from 5 to 7.5 g per day, showing a significant improvement in vision, with patients experiencing a gain of at least 15 letters [85].

2.5. Curcumin

Curcumin (diferuloylmethane) is the main bioactive component of the popular Indian spice turmeric (*Curcuma longa*) and is a water-insoluble yellowish-orange colored polyphenol. It is isolated from the dried rhizome of *Curcuma longa* L. which belongs to the *Zingiberaceae* family [86,87]. Belonging to the group of phytochemicals, curcumin is considered a bioactive molecule with pleiotropic effects, demonstrating antioxidative, anti-inflammatory, antimicrobial, antimutagenic, and antiproliferative properties [88–90]. The mechanism by which curcumin induces its effects is still not fully understood, but it is considered as a nutraceutical substance for the treatment of some chronic diseases such as diabetes, neurodegenerative diseases, pulmonary infectious, rheumatism, and oncological diseases [91,92].

Curcumin exhibits promising therapeutic potential in treating various eye conditions, including diabetic retinopathy, chronic anterior uveitis, glaucoma, dry eye syndrome, and AMD [93]. Its mechanisms of action involve reducing apoptosis rates in RPE cells and decreasing overall inflammation by downregulating genes associated with inflammation in AMD [93,94]. Specifically, curcumin's biological, nutraceutical, and pharmaceutical properties has been linked to its ability to lower levels of TNF-α and proinflammatory interleukins (IL-1, IL-6, IL-8) [86]. Moreover, curcumin protects RPE against oxidative stress and inflammation by activating Nrf2/HO-1 signaling and modulating the ERK pathway, offering potential therapeutic benefits for diabetic retinopathy and AMD [86] (Figure 2).

These positive effects make curcumin a candidate for treating inflammatory and degenerative retinal eye conditions [86], but research studies on the efficacy of curcumin are mainly limited to in vitro and in vivo studies.

In an animal study, Mandal et al. observed significant retinal neuroprotection in rats fed diets supplemented with curcumin (0.2% in diet) for 2 weeks [95]. This effect was

attributed to curcumin's ability to downregulate cellular inflammatory genes and inhibit NF-κB activation [95]. Another study found that curcumin reduced the expression of free radicals and gene expression of oxidative biomarkers such as superoxide dismutase (SOD), glutathione, and malondialdehyde [96]. In this study, AMD was modeled by inducing aging in RPE cells with pulsed H_2O_2, demonstrating that curcumin led to decreased apoptosis and thus higher cell viability [96]. In addition, a study demonstrated that curcumin has post-transcriptional regulatory effects and can induce gene silencing [97]. Curcumin was also observed to both up-regulate and down-regulate specific microRNAs (miRNAs) that could play a role in regulating the antioxidant system [97].

Curcumin, as a PPAR-γ agonist, may help slow AMD progression by reducing microglia's proinflammatory effects. Saberi et al. found that curcumin activates PPAR-γ, leading to lower production of matrix metalloproteinases (MMPs), particularly MMP-9, which is involved in AMD pathogenesis by promoting RPE cell migration and extracellular matrix degradation [98]. However, exploiting the biomedical potential of curcumin is difficult due to its limited solubility and poor oral bioavailability. The main factors contributing to the low levels of curcumin in plasma and tissues seem to be attributed to its poor absorption, rapid metabolism, and rapid systemic elimination [99]. For this reason, there has been a growing interest on nanoparticles and liposomes to enhance curcumin's bioavailability [100].

In conclusion, curcumin has good tolerance and does not exhibit dose-limiting toxicity and it could be considered safe even at high doses (6 g/day orally for 4–7 weeks) in humans [101].

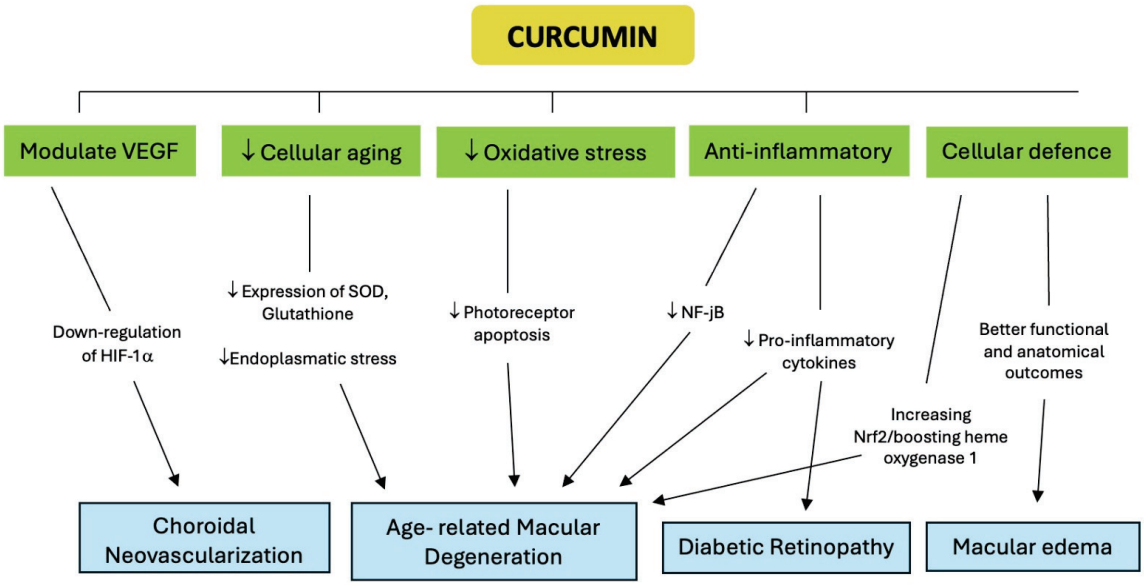

Figure 2. Beneficial mechanisms of action of curcumin on retinal diseases.

2.6. Saffron

Saffron is a spice obtained from *Crocus sativus* L., a plant of the Iridaceae family. Since ancient times, it has been used as an herbal medicine and as a coloring and flavoring spice. Saffron and its major constituents, such as crocetin, crocin, picrocin, and safranal, are natural carotenoids with antioxidant, anti-inflammatory, and neuroprotective effects [102]. These neuroprotective effects have been studied in neurodegenerative diseases such as Alzheimer's and Parkinson's [103] and in ocular diseases including AMD, glaucoma, and other retinal diseases. An increasing number of studies have explored the effects and mechanistic pathways of saffron and its compounds to assess their potential therapeutic use in ocular diseases. Most studies showed that saffron has a strong antioxidant activity, due to its carotenoids, especially crocin, protecting biomolecules from free radicals [104]. Additionally, saffron components exhibited anti-inflammatory and antiapoptotic effects

possibly by inhibiting apoptosis mediated by caspase after retinal damage [105,106]. Moreover, crocin and crocetin are also known to enhance oxygen diffusion and improve ocular blood flow in the retina and choroid, which are crucial factors in AMD progression [107]. Considering AMD patients, six clinical studies assessed vision-related parameters after oral saffron supplementation [108–113]. All these studies showed significant improvement in visual acuity across different saffron dosages (ranging from 20 to 50 mg daily), even with short-term supplementation of around three months [108–113].

It is not possible to make a direct quantitative comparison because the above-mentioned studies had differences in formulation, dosage, intervention length, test methods, and outcome measures.

Longer-term data are available from only two non-randomized control trials, both showing improvements within three months of saffron supplementation (20 mg daily), with a stabilization over time [109,110].

2.7. Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) is a neurohormone produced by the pineal gland in the human brain. Is a ubiquitous biomolecule in nature, also produced by other species of animals, plants, and microorganisms [114]. Additionally, this hormone is synthesized in human ocular tissues, including the retina and ciliary body, which have specific melatonin receptors [115]. Both melatonin and melanopsin, a regulator of melatonin synthesis in the eyes, are produced in the human lens [116]. Melatonin controls the circadian rhythm and sleep–wake regulation. Furthermore, it regulates various physiological functions including immune response, retinal physiology, sexual behavior, body temperature, and aging processes [117]. Melatonin acts as a scavenger for free radicals, playing a crucial role in detoxifying ROS. This protective action not only shields cells from damage caused by oxidative stress but also regulates the transcription of antioxidant genes through Nrf2 activation [118,119]. Additionally, melatonin influences several other molecular pathways including inflammation, apoptosis, angiogenesis, and autophagy [120]. Recent studies suggest that melatonin may decrease inflammation, oxidative stress, and apoptosis in the retina and increase viability of RPE cells as well as of photoreceptors, indicating its potential role in preventing and treating AMD [121,122]. One study reported that AMD patients have lower levels of nocturnal melatonin production compared to age-matched controls [123]. This could suggest that the decreased synthesis of melatonin may contribute to oxidative stress and the onset of AMD. Moreover, another study showed that the daily use of 3 mg of exogenous melatonin, for 3 months, in one hundred patients with AMD could protect the retina and delay AMD onset [124].

Phagocytic and autophagic processes in RPE cells are influenced by circadian rhythms [125], but the specific role of melatonin is not yet clear. Some studies indicated that melatonin may regulate photoreceptor phagocytosis [126], while others did not confirm this relationship. Retinal circadian clocks protect photoreceptors by reducing melatonin secretion during light exposure [127].

Melatonin also protects RPE cells from light-induced apoptosis through the aggregation of melanosomes and the absorption of light by melanin, which acts as an antioxidant [128]. Melatonin plays a crucial role in protecting the blood-retinal barrier in AMD-associated neovascularization, triggered by the accumulation of ROS [129]. This molecule may reduce the levels of VEGF and nitric oxide in the retina [129], thus preventing degradation of the blood–retinal barrier. Such action of melatonin suggests its potential effect in the treatment of AMD and suggests that its administration, especially in combination with antiangiogenic agents, could increase the efficacy of AMD therapy [119].

3. Role of Diet and Food Intake

In the last few years, in addition to all the aforesaid molecules (Table 1), there has been a high interest in the role of diet and food intake since they are considered as modifiable factors for preventing or slowing the progression of AMD [130,131].

Table 1. Overview of the main effects of the discussed oral supplement compounds for the management of age-related macular degeneration.

Active Compounds	Daily Therapeutic Dosage	Prescription Duration	Effects	Bioavailability	Adverse Effect and Contraindications	References	Clinical Trial Number
Lutein	1–20 mg	Weeks–months	Antioxidant activity Improvement of visual function	Moderate	No adverse effects demonstrated	[23,31–33]	NCT00345176 NCT00763659 NCT01042860 ISRCTN94557601
Zeaxanthin	2–10 mg	Weeks–months	Antioxidant activity Improvement of visual function	Moderate	May give the skin a golden-yellow color in people with fair complexions	[10,23,31,33]	NCT01527435 NCT00345176 NCT00763659 ISRCTN94557601
Astaxanthin	4–20 mg	Months	Antioxidant activity Anti-inflammatory effects	Low/Moderate	Minimal toxicity	[37,42]	
Vitamin A	700–900 mcg	Weeks	Antioxidant activity	Moderate/High	Hypervitaminosis, with possible permanent damage to the liver and spleen	[4]	NCT00000145
Vitamin C	100–500 mg	Months	Antioxidant activity	High	Digestive problems, vomiting, diarrhea, gastritis; may increase the risk of kidney stones in men	[23,33]	NCT00345176 ISRCTN94557601
Vitamin E	400–500 UI	Months	Antioxidant activity	High	Digestive problems, vomiting, nausea; may increase blood pression and reduce thyroid hormones	[23,33]	NCT00345176 ISRCTN94557601
Zinc	2–80 mg	Weeks–months	Antioxidant activity	High	Vomiting, nausea or diarrhea, irritability, drowsiness, anemia, dizziness; may cause copper deficiency anemia	[23,33]	NCT00345176 ISRCTN94557601
Selenium	50–200 μg	Weeks–months	Antioxidant activity	High	Digestive problems, vomiting, diarrhea, nausea	[50,72]	
Eicosapentaenoic acid Docosahexaenoic acid	350 mg–7.5 g	Weeks–months	Anti-inflammatory effects Antioxidant properties Anti-angiogenic and anti-vasoproliferative effects Neuroprotective effects	Moderate	May increase the risk of bleeding	[23,31]	NCT00345176 NCT00763659
Curcumin	500 mg–3 g	3–4 weeks	Possible prevention of retinal pigment epithelium cell death Anti-inflammatory effects Antioxidant activity	Very Low	Minimal toxicity, predominantly gastrointestinal upsets; may interfere with antibiotics, cardiovascular drugs, anticancer drugs, and antidepressants	[86]	
Saffron	20–30 mg	Weeks–months	Anti-inflammatory effects Antioxidant activity	Moderate	Mood disorders, lethal dose of approximately 20 g; May interfere with cardiovascular drugs	[9,111]	ACTRN12612000729820 IRCT2012052198 20N1
Melatonin	3–20 mg	Weeks–months	Antioxidant activity Anti-inflammatory effect Possible increase in RPE cell viability	Moderate	Drowsiness; may enhance sedative effects of some drugs such as benzodiazepines	[119,124]	

Recent studies have highlighted the relationship between the Mediterranean diet and AMD [132–134]. The Mediterranean diet is characterized by a high intake of vegetables, fruits, legumes, grains, and nuts; moderate consumption of poultry, dairy and fish; limited consumption of red meat; and low to moderate amounts of red wine may be consumed.

Olive oil is used instead of butter as the main condiment fat. High adherence to this dietary pattern has been associated with a lower risk of developing AMD [133,134] and has also been shown to be beneficial for reducing the progression of late AMD [135]. This dietary approach could potentially mitigate oxidative stress and inflammation and provide a shield against AMD progression. The consumption of specific foods also appears to protect against the risk of AMD, such as vegetables rich in carotenoids, especially dark green leafy vegetables [22,135], and fatty fish containing omega-3 fatty acids [82,136]. Conversely, high consumption of foods like animal fats containing omega-6 fatty acids [137] and red and processed meat [138] should be minimized to reduce the risk of AMD progression (Figure 3).

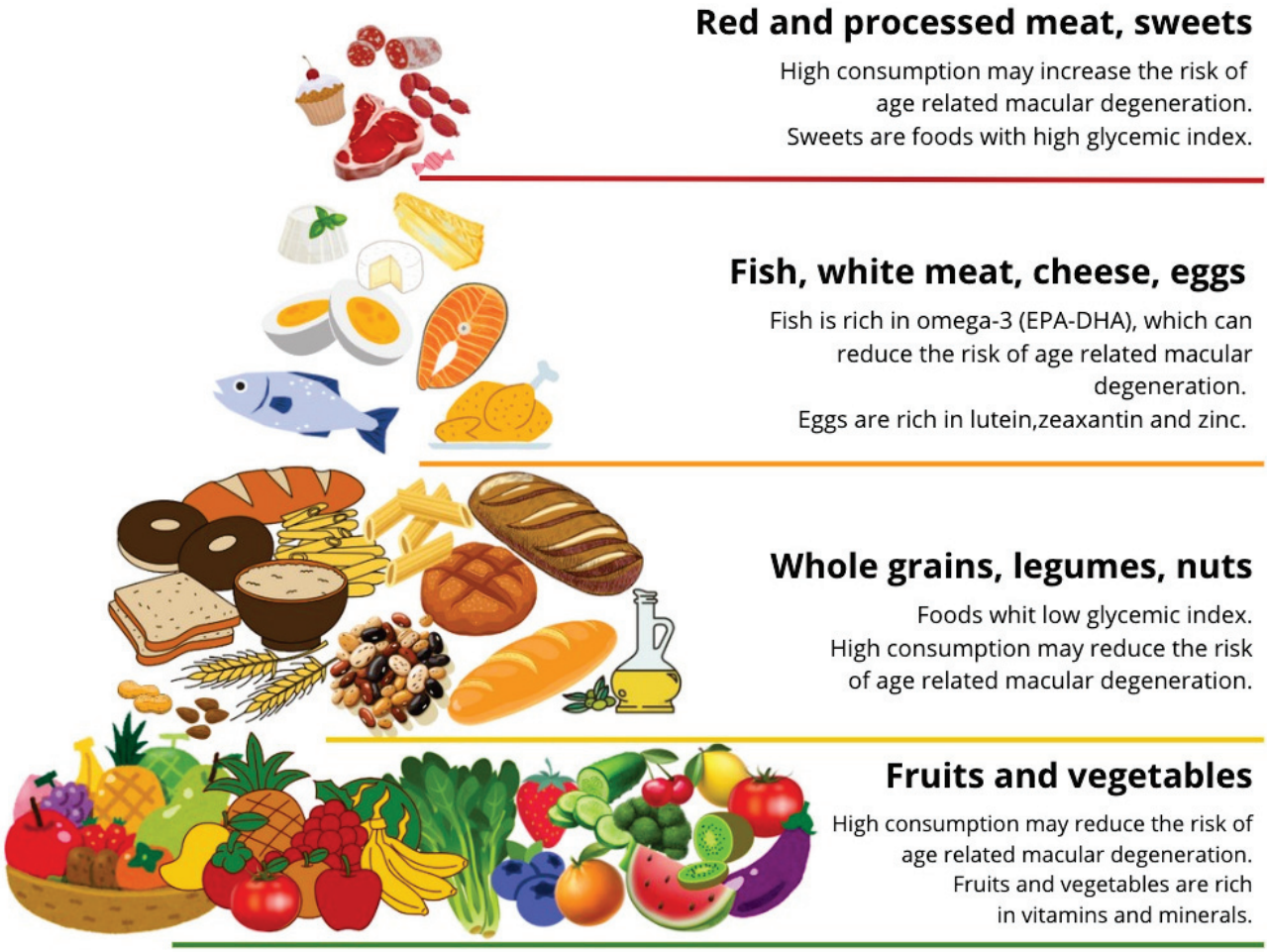

Figure 3. Simplified schematic representation of the food pyramid showing the possible role of food intake on age-related macular degeneration.

Table 2 provides a more in-depth overview of the potential role of diet and food intake in AMD.

Encouraging dietary improvements could help reduce AMD risk by focusing on nutrient-rich foods and avoiding those that cause oxidative damage. Providing nutritional guidance alongside standard treatments could be important for managing AMD effectively.

Table 2. Overview of the possible role of diet and food intake in age-related macular degeneration.

Dietary Factor	Possible Effects on AMD	General Considerations	References
Mediterranean diet	Lower risk of developing AMD Lower risk of AMD progression	High consumption of fruits, vegetables, legumes, whole grains and nuts; moderate consumption of fish, poultry and dairy; limited consumption of red meat; olive oil used as main fat; low to moderate amounts of red wine	[131–134]
Western diet	Increased AMD prevalence	Higher intake of red meat, processed meat, high-fat dairy products, fried potatoes, refined grains	[135]
High glycemic index diets	Increased risk of early AMD	Glycemic index is a measure that ranks carbohydrate-containing foods based on their impact on blood sugar levels over a 2 h period. Pure glucose is assigned the reference GI of 100. High consumption of foods with high GI (>70): white bread, potatoes, white rice, cereals, honey, and refined sugar Low consumption of foods with low GI (<55): whole fruit and vegetables, whole wheat bread, pasta, oats, bran, legumes, milk, and yoghurt	[139,140]
Vegetables and fruits	Lower risk of AMD	200 g per day of vegetables, in particular dark green leafy vegetables (broccoli, spinach. . .) and fruit two times per day	[22,48]
Fish and omega-3	Reduced risk of AMD	One or more servings of fish per week	[48,136,137]
Red and processed meat	Increased risk of developing AMD	High consumption especially of processed meat (salami or continental sausages)	[138]
Alcohol	Higher risk of development AMD	More than two drinks a day	[141,142]

AMD: Age-related macular degeneration; GI: glycemic index.

4. Discussion

This review focused on the preventive qualities and effects of several oral supplements as well as food intake for managing AMD. By shielding RPE cells from oxidative stress and preventing them from apoptosis, most of the oral supplements examined in this review provide therapeutic benefits through primarily antioxidant actions. While these supplements cannot replace traditional medical and surgical treatment, they can have a positive synergistic effect that boosts the efficacy of gold-standard therapies and aids in stabilizing the patient's general state of health.

In fact, the growing use of these oral supplements in clinical practice for the management of AMD has aroused considerable interest on the part of researchers to better understand their potential beneficial effects for the treatment of this ocular disease, adding them to conventional therapy characterized by intravitreal anti-VEGF drugs.

Considering oral supplements, all have shown substantially protective activities towards the retina in case of AMD, both in reducing the risk of development and progression of the disease. Furthermore, except for curcumin, all of these substances have moderate to high bioavailability when taken orally, with a good safety and tolerability profile. In fact, excluding curcumin and zinc, which showed a greater interaction with other drugs and a greater onset of undangerous gastrointestinal disorders, all molecules have demonstrated minimal toxicity in the clinical studies published in the literature.

While lutein and zeaxanthin are well recognized as retinal protective molecules, having already been included in the AREDS2 study [23], of particular interest are certainly astaxanthin, EPA, and DHA, which have shown very promising effects thanks to their anti-inflammatory, anti-angiogenic, anti-vasoproliferative and neuroprotective properties. In particular, all these molecules can exert their beneficial effects by modulating the immune

response, thus reducing the production of pro-inflammatory cytokines and leukocyte activation, together with the increase of anti-inflammatory derivatives [76,77].

In addition to oral supplements, diet and food intake have also recently attracted increasing interest in the ophthalmology field, in particular in the clinical management of degenerative diseases such as AMD. In fact, several studies in the literature have demonstrated the protective effects of some foods, such as fish, fruit, and vegetables, and the harmful effects of others, such as red and processed meat, alcohol, and all foods with a high glycemic index. However, more scientific evidence is needed to better establish the relationship between AMD and diet, and to better understand the potential benefits of healthy food for the management and the prevention of this ocular disease.

The narrative and non-systematic nature of this review, together with the fact that it was composed using only one scientific database (PubMed), could be considered limitations of this study. Furthermore, only some of the main oral supplements that are currently used in clinical practice to control AMD have been reviewed in this study.

5. Conclusions

The integrative use of antioxidants, vitamins, organic compounds, and micronutrients, along with a balanced and healthy diet, may be beneficial for the treatment of AMD, as well as glaucoma and other ocular disorders [143–145].

However, effective oral supplementation strategies are challenging to design due to a number of limiting factors, including the complexity of the anatomy and tissues of the visual system, the timing of patient enrollment in clinical trials, the lack of valid endpoints, and our incomplete understanding of the underlying molecular causes of neurodegenerative diseases, including AMD.

For this reason, more investigations and even large-scale multicenter clinical trials are continuously required to confirm the efficacy of these oral supplements and to gain a deeper understanding of their mechanisms of action to fully utilize their therapeutical properties as adjuvant therapeutic alternatives in treatment regimens for AMD, in addition to the promotion of a healthy diet, given the potential benefits it can bring to AMD patients.

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References

1. Carnevali, A.; Mastropasqua, R.; Gatti, V.; Vaccaro, S.; Mancini, A.; D'aloisio, R.; Lupidi, M.; Cerquaglia, A.; Sacconi, R.; Borrelli, E.; et al. Optical Coherence Tomography Angiography in Intermediate and Late Age-Related Macular Degeneration: Review of Current Technical Aspects and Applications. *Appl. Sci.* **2020**, *10*, 8865. [CrossRef]
2. Fleckenstein, M.; Schmitz-Valckenberg, S.; Chakravarthy, U. Age-Related Macular Degeneration: A Review. *JAMA* **2024**, *331*, 147–157. [CrossRef] [PubMed]
3. Lim, L.S.; Mitchell, P.; Seddon, J.M.; Holz, F.G.; Wong, T.Y. Age-related macular degeneration. *Lancet* **2012**, *379*, 1728–1738. [CrossRef] [PubMed]
4. Age-Related Eye Disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch. Ophthalmol.* **2001**, *119*, 1417–1436. [CrossRef] [PubMed]
5. Klein, R.; Klein, B.E.; Linton, K.L. Prevalence of age-related maculopathy: The Beaver Dam Eye Study. *Ophthalmology* **1992**, *99*, 933–943. [CrossRef] [PubMed]
6. Semenova, Y.; Bjørklund, G. Antioxidants and neurodegenerative eye disease. *Crit. Rev. Food Sci. Nutr.* **2023**, 1–19. [CrossRef] [PubMed]

7. Zhang, Z.; Gu, Q.; Chen, L.; Yuan, D.; Gu, X.; Qian, H.; Xie, P.; Liu, Q.; Hu, Z. Selective microRNA expression of exosomes from retinal pigment epithelial cells by oxidative stress. *Vis. Res.* **2024**, *220*, 108388. [CrossRef] [PubMed]
8. Tan, W.; Zou, J.; Yoshida, S.; Jiang, B.; Zhou, Y. The Role of Inflammation in Age-Related Macular Degeneration. *Int. J. Biol. Sci.* **2020**, *16*, 2989–3001. [CrossRef] [PubMed]
9. Broadhead, G.K.; Grigg, J.; McCluskey, P.J.; Hong, T.; Schlub, T.E.; Chu, E.; Chang, A.A. Saffron therapy for the ongoing treatment of age-related macular degeneration. *BMJ Open Ophthalmol.* **2024**, *9*, e001399. [CrossRef]
10. Brown, G.C.; Brown, M.M.; Gierhart, D.; Olk, R.J. Prevention Surpasses Treatment: 5-year Follow-Up, Cost-Utility, and Cost-Benefit of Zeaxanthin Therapy for Neovascular Age-Related Macular Degeneration. *Ophthalmol. Ther.* **2023**, *12*, 2583–2608. [CrossRef]
11. Evans, J.R.; Lawrenson, J.G. Antioxidant vitamin and mineral supplements for preventing age-related macular degeneration. *Cochrane Database Syst. Rev.* **2017**, *7*, CD000253. [CrossRef] [PubMed]
12. Mrowicka, M.; Mrowicki, J.; Kucharska, E.; Majsterek, I. Lutein and Zeaxanthin and Their Roles in Age-Related Macular Degeneration—Neurodegenerative Disease. *Nutrients* **2022**, *14*, 827. [CrossRef] [PubMed]
13. Li, L.H.; Lee, J.C.-Y.; Leung, H.H.; Lam, W.C.; Fu, Z.; Lo, A.C.Y. Lutein Supplementation for Eye Diseases. *Nutrients* **2020**, *12*, 1721. [CrossRef] [PubMed]
14. Eisenhauer, B.; Natoli, S.; Liew, G.; Flood, V.M. Lutein and Zeaxanthin-Food Sources, Bioavailability and Dietary Variety in Age-Related Macular Degeneration Protection. *Nutrients* **2017**, *9*, 120. [CrossRef] [PubMed]
15. Carpentier, S.; Knaus, M.; Suh, M. Associations between lutein, zeaxanthin, and age-related macular degeneration: An overview. *Crit. Rev. Food Sci. Nutr.* **2009**, *49*, 313–326. [CrossRef] [PubMed]
16. Richer, S.; Stiles, W.; Statkute, L.; Pulido, J.; Frankowski, J.; Rudy, D.; Pei, K.; Tsipursky, M.; Nyland, J. Double-masked, placebo-controlled, randomized trial of lutein and antioxidant supplementation in the intervention of atrophic age-related macular degeneration: The Veterans LAST study (Lutein Antioxidant Supplementation Trial). *Optometry* **2004**, *75*, 216–230. [CrossRef] [PubMed]
17. Falsini, B.; Piccardi, M.; Iarossi, G.; Fadda, A.; Merendino, E.; Valentini, P. Influence of short-term antioxidant supplementation on macular function in age-related maculopathy. A pilot study including electrophysiologic assessment. *Ophthalmology* **2003**, *110*, 51–60, discussion 61. [CrossRef] [PubMed]
18. Sujak, A.; Gabrielska, J.; Grudziński, W.; Borc, R.; Mazurek, P.; Gruszecki, W.I. Lutein and zeaxanthin as protectors of lipid membranes against oxidative damage: The structural aspects. *Arch. Biochem. Biophys.* **1999**, *371*, 301–307. [CrossRef] [PubMed]
19. Bone, R.A.; Ruiz, C.A.; Landrum, J.T.; Guerra, L.H. Lutein and zeaxanthin dietary supplements raise macular pigment density and serum concentrations of these carotenoids in humans. *J. Nutr.* **2003**, *133*, 992–998. [CrossRef]
20. Junghans, A.; Sies, H.; Stahl, W. Macular pigments lutein and zeaxanthin as blue light filters studied in liposomes. *Arch. Biochem. Biophys.* **2001**, *391*, 160–164. [CrossRef]
21. Krinsky, N.I.; Landrum, J.T.; Bone, R.A. Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. *Annu. Rev. Nutr.* **2003**, *23*, 171–201. [CrossRef] [PubMed]
22. Seddon, J.M.; Ajani, U.A.; Sperduto, R.D.; Hiller, R.; Blair, N.; Burton, T.C.; Farber, M.D.; Gragoudas, E.S.; Haller, J.; Miller, D.T.; et al. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. *JAMA* **1994**, *272*, 1413–1420. [CrossRef] [PubMed]
23. Age-Related Eye Disease Study 2 Research Group. Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: The Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. *JAMA* **2013**, *309*, 2005–2015. [CrossRef] [PubMed]
24. Tanvetyanon, T.; Bepler, G. Beta-carotene in multivitamins and the possible risk of lung cancer among smokers versus former smokers: A meta-analysis and evaluation of national brands. *Cancer* **2008**, *113*, 150–157. [CrossRef] [PubMed]
25. Wang, J.J.; Buitendijk, G.H.; Rochtchina, E.; Lee, K.E.; Klein, B.E.; van Duijn, C.M.; Flood, V.M.; Meuer, S.M.; Attia, J.; Myers, C.; et al. Genetic susceptibility, dietary antioxidants, and long-term incidence of age-related macular degeneration in two populations. *Ophthalmology* **2013**, *121*, 667–675. [CrossRef] [PubMed]
26. Liu, R.; Wang, T.; Zhang, B.; Qin, L.; Wu, C.; Li, Q.; Ma, L. Lutein and zeaxanthin supplementation and association with visual function in age-related macular degeneration. *Investig. Ophthalmol. Vis. Sci.* **2014**, *56*, 252–258. [CrossRef] [PubMed]
27. The Carmis Study Group; Piermarocchi, S.; Saviano, S.; Parisi, V.; Tedeschi, M.; Panozzo, G.; Scarpa, G.; Boschi, G.; Giudice, G.L.; Sartore, M.; et al. Carotenoids in Age-related Maculopathy Italian Study (CARMIS): Two-year results of a randomized study. *Eur. J. Ophthalmol.* **2012**, *22*, 216–225. [CrossRef]
28. Bartlett, H.E.; Eperjesi, F. Effect of lutein and antioxidant dietary supplementation on contrast sensitivity in age-related macular disease: A randomized controlled trial. *Eur. J. Clin. Nutr.* **2007**, *61*, 1121–1127. [CrossRef] [PubMed]
29. Ma, L.; Yan, S.-F.; Huang, Y.-M.; Lu, X.-R.; Qian, F.; Pang, H.-L.; Xu, X.-R.; Zou, Z.-Y.; Dong, P.-C.; Xiao, X.; et al. Effect of lutein and zeaxanthin on macular pigment and visual function in patients with early age-related macular degeneration. *Ophthalmology* **2012**, *119*, 2290–2297. [CrossRef] [PubMed]
30. Weigert, G.; Kaya, S.; Pemp, B.; Sacu, S.; Lasta, M.; Werkmeister, R.M.; Dragostinoff, N.; Simader, C.; Garhöfer, G.; Schmidt-Erfurth, U.; et al. Effects of lutein supplementation on macular pigment optical density and visual acuity in patients with age-related macular degeneration. *Investig. Opthalmol. Vis. Sci.* **2011**, *52*, 8174–8178. [CrossRef]

31. Dawczynski, J.; Jentsch, S.; Schweitzer, D.; Hammer, M.; Lang, G.E.; Strobel, J. Long term effects of lutein, zeaxanthin and omega-3-LCPUFAs supplementation on optical density of macular pigment in AMD patients: The LUTEGA study. *Graefe's Arch. Clin. Exp. Ophthalmol.* **2013**, *251*, 2711–2723. [CrossRef] [PubMed]
32. Murray, I.J.; Makridaki, M.; van der Veen, R.L.P.; Carden, D.; Parry, N.R.A.; Berendschot, T.T.J.M. Lutein supplementation over a one-year period in early AMD might have a mild beneficial effect on visual acuity: The CLEAR Study. *Investig. Opthalmol. Vis. Sci.* **2013**, *54*, 1781–1788. [CrossRef] [PubMed]
33. Beatty, S.; Chakravarthy, U.; Nolan, J.M.; Muldrew, K.A.; Woodside, J.V.; Denny, F.; Stevenson, M.R. Secondary outcomes in a clinical trial of carotenoids with coantioxidants versus placebo in early age-related macular degeneration. *Ophthalmology* **2013**, *120*, 600–606. [CrossRef] [PubMed]
34. Ambati, R.R.; Phang, S.M.; Ravi, S.; Aswathanarayana, R.G. Astaxanthin: Sources, extraction, stability, biological activities and its commercial applications-a review. *Mar. Drugs* **2014**, *12*, 128–152. [CrossRef] [PubMed]
35. Yuan, J.; Peng, J.; Yin, K.; Wang, J. Potential health-promoting effects of astaxanthin: A high-value carotenoid mostly from microalgae. *Mol. Nutr. Food Res.* **2010**, *55*, 150–165. [CrossRef] [PubMed]
36. Fakhri, S.; Abbaszadeh, F.; Dargahi, L.; Jorjani, M. Astaxanthin: A mechanistic review on its biological activities and health benefits. *Pharmacol. Res.* **2018**, *136*, 1–20. [CrossRef] [PubMed]
37. Giannaccare, G.; Pellegrini, M.; Senni, C.; Bernabei, F.; Scorcia, V.; Cicero, A.F.G. Clinical Applications of Astaxanthin in the Treatment of Ocular Diseases: Emerging Insights. *Mar. Drugs* **2020**, *18*, 239. [CrossRef] [PubMed]
38. Naguib, Y.M.A. Antioxidant Activities of Astaxanthin and related carotenoids. *J. Agric. Food Chem.* **2000**, *48*, 1150–1154. [CrossRef]
39. Lee, S.J.; Bai, S.K.; Lee, K.S.; Namkoong, S.; Na, H.J.; Ha, K.S.; Han, J.A.; Yim, S.V.; Chang, K.; Kwon, Y.G.; et al. Astaxanthin inhibits nitric oxide production and inflammatory gene expression by suppressing I(kappa)B kinase-dependent NF-kappaB activation. *Mol. Cells* **2003**, *16*, 97–105. [CrossRef]
40. Kidd, P. Astaxanthin, cell membrane nutrient with diverse clinical benefits and anti-aging potential. *Altern. Med. Rev.* **2011**, *16*, 355–364.
41. Parisi, V.; Tedeschi, M.; Gallinaro, G.; Varano, M.; Saviano, S.; Piermarocchi, S.; CARMIS Study Group. Carotenoids and antioxidants in age-related maculopathy italian study: Multifocal electroretinogram modifications after 1 year. *Ophthalmology* **2008**, *115*, 324–333.e2. [CrossRef] [PubMed]
42. Izumi-Nagai, K.; Nagai, N.; Ohgami, K.; Satofuka, S.; Ozawa, Y.; Tsubota, K.; Ohno, S.; Oike, Y.; Ishida, S. Inhibition of choroidal neovascularization with an anti-inflammatory carotenoid astaxanthin. *Investig. Ophthalmol. Vis. Sci.* **2008**, *49*, 1679–1685. [CrossRef] [PubMed]
43. Cicero, A.F.; Colletti, A. Effects of Carotenoids on Health: Are All the Same? Results from Clinical Trials. *Curr. Pharm. Des.* **2017**, *23*, 2422–2427. [CrossRef] [PubMed]
44. Gaynes, B.I. AREDS misses on safety. *Arch. Ophthalmol.* **2003**, *121*, 416–417. [CrossRef]
45. Bates, C.J. Vitamin A. *Lancet* **1995**, *345*, 31–35. [CrossRef]
46. Schmidl, D.; Garhöfer, G.; Schmetterer, L. Nutritional supplements in age-related macular degeneration. *Acta Ophthalmol.* **2015**, *93*, 105–121. [CrossRef] [PubMed]
47. Goldberg, J.; Flowerdew, G.; Smith, E.; Brody, J.A.; Tso, M.O. Factors associated with age-related macular degeneration. An analysis of data from the first National Health and Nutrition Examination Survey. *Am. J. Epidemiol.* **1988**, *128*, 700–710. [CrossRef]
48. de Koning-Backus, A.P.; Buitendijk, G.H.; Kiefte-de Jong, J.C.; Colijn, J.M.; Hofman, A.; Vingerling, J.R.; Haverkort, E.B.; Franco, O.H.; Klaver, C.C. Intake of Vegetables, Fruit, and Fish is Beneficial for Age-Related Macular Degeneration. *Am. J. Ophthalmol.* **2019**, *198*, 70–79. [CrossRef] [PubMed]
49. Delcourt, C.; Cristol, J.-P.; Tessier, F.; Léger, C.L.; Descomps, B.; Papoz, L. Age-related macular degeneration and antioxidant status in the POLA study. *Arch. Ophthalmol.* **1999**, *117*, 1384–1390. [CrossRef]
50. Monsen, E.R. Dietary reference intakes for the antioxidant nutrients: Vitamin C, vitamin E, selenium, and carotenoids. *J. Am. Diet. Assoc.* **2000**, *100*, 637–640. [CrossRef]
51. Organisciak, D.; Jiang, Y.; Wang, H.; Bicknell, I. The protective effect of ascorbic-acid in retinal light damage of rats exposed to intermittent light. *Investig. Ophthalmol. Vis. Sci.* **1990**, *31*, 1195–1202.
52. SanGiovanni, J.P.; Chew, E.Y.; Clemons, T.E.; Ferris, F.L., 3rd; Gensler, G.; Lindblad, A.S.; Milton, R.C.; Seddon, J.M.; Sperduto, R.D. The relationship of dietary carotenoid and vitamin A, E, and C intake with age-related macular degeneration in a case-control study: AREDS Report No. 22. *Arch. Ophthalmol.* **2007**, *125*, 1225–1232.
53. Eye Disease Case-Control Study Group. Antioxidant status and neovascular age-related macular degeneration. *Arch. Ophthalmol.* **1993**, *111*, 104–109. [CrossRef] [PubMed]
54. Olmedilla, B.; Granado, F.; Blanco, I.; Vaquero, M. Lutein, but not α-tocopherol, supplementation improves visual function in patients with age-related cataracts: A 2-y double-blind, placebo-controlled pilot study. *Nutrition* **2003**, *19*, 21–24. [CrossRef]
55. Alvarez, R.A.; Liou, G.I.; Fong, S.L.; Bridges, C.D. Levels of alpha- and gamma-tocopherol in human eyes: Evaluation of the possible role of IRBP in intraocular alpha-tocopherol transport. *Am. J. Clin. Nutr.* **1987**, *46*, 481–487. [CrossRef] [PubMed]
56. Stephens, R.; Negi, D.; Short, S.; van Kuijk, F.; Dratz, E.; Thomas, D. Vitamin E distribution in ocular tissues following long-term dietary depletion and supplementation as determined by microdissection and gas chromatography-mass spectrometry. *Exp. Eye Res.* **1988**, *47*, 237–245. [CrossRef]

57. West, S.; Vitale, S.; Hallfrisch, J.; Muñoz, B.; Muller, D.; Bressler, S.; Bressler, N.M. Are antioxidants or supplements protective for age-related macular degeneration? *Arch. Ophthalmol.* **1994**, *112*, 222–227. [CrossRef]
58. Mares-Perlman, J.A.; Brady, W.E.; Klein, R.; Klein, B.E.K.; Bowen, P.; Stacewicz-Sapuntzakis, M.; Palta, M. Serum antioxidants and age-related macular degeneration in a population-based case-control study. *Arch. Ophthalmol.* **1995**, *113*, 1518–1523. [CrossRef]
59. Thurnham, D.I. Macular zeaxanthins and lutein—A review of dietary sources and bioavailability and some relationships with macular pigment optical density and age-related macular disease. *Nutr. Res. Rev.* **2007**, *20*, 163–179. [CrossRef]
60. Taylor, H.R.; Tikellis, G.; Robman, L.D.; McCarty, C.A.; McNeil, J.J. Vitamin E supplementation and macular degeneration: Randomised controlled trial. *BMJ* **2002**, *325*, 11. [CrossRef]
61. King, J.C.; Shames, D.M.; Woodhouse, L.R. Zinc homeostasis in humans. *J. Nutr.* **2000**, *130* (Suppl. 5S), 1360S–1366S. [CrossRef] [PubMed]
62. Blasiak, J.; Pawlowska, E.; Chojnacki, J.; Szczepanska, J.; Chojnacki, C.; Kaarniranta, K. Zinc and Autophagy in Age-Related Macular Degeneration. *Int. J. Mol. Sci.* **2020**, *21*, 4994. [CrossRef] [PubMed]
63. Duan, M.; Li, T.; Liu, B.; Yin, S.; Zang, J.; Lv, C.; Zhao, G.; Zhang, T. Zinc nutrition and dietary zinc supplements. *Crit. Rev. Food Sci. Nutr.* **2021**, *63*, 1277–1292. [CrossRef] [PubMed]
64. Fosmire, G.J. Zinc toxicity. *Am. J. Clin. Nutr.* **1990**, *51*, 225–227. [CrossRef] [PubMed]
65. McCall, K.A.; Huang, C.-C.; Fierke, C.A. Function and mechanism of zinc metalloenzymes. *J. Nutr.* **2000**, *130* (Suppl. 5S), 1437S–1446S. [CrossRef] [PubMed]
66. Ripps, H.; Chappell, R.L. Zinc's functional significance in the vertebrate retina. *Mol. Vis.* **2014**, *20*, 1067–1074. [PubMed]
67. Leung, E.; Landa, G. Update on current and future novel therapies for dry age-related macular degeneration. *Expert Rev. Clin. Pharmacol.* **2013**, *6*, 565–579. [CrossRef]
68. Newsome, D.A.; Swartz, M.; Leone, N.C.; Elston, R.C.; Miller, E. Oral zinc in macular degeneration. *Arch. Ophthalmol.* **1988**, *106*, 192–198. [CrossRef]
69. Tittl, M.; Reitner, A.; Meisinger, V.; Stur, M. Oral zinc and the second eye in age-related macular degeneration. *Investig. Ophthalmol. Vis. Sci.* **1996**, *37*, 1225–1235. [CrossRef]
70. Cho, E.; Stampfer, M.J.; Seddon, J.M.; Hung, S.; Spiegelman, D.; Rimm, E.B.; Willett, W.C.; Hankinson, S.E. Prospective study of zinc intake and the risk of age-related macular degeneration. *Ann. Epidemiol.* **2001**, *11*, 328–336. [CrossRef]
71. Wong, P.; Markey, M.; Rapp, C.M.; Darrow, R.M.; Ziesel, A.; Organisciak, D. Enhancing the efficacy of AREDS antioxidants in light-induced retinal degeneration. *Mol. Vis.* **2017**, *23*, 718–739. [PubMed]
72. Ursini, F.; Bindoli, A. The role of selenium peroxidases in the protection against oxidative damage of membranes. *Chem. Phys. Lipids* **1987**, *44*, 255–276. [CrossRef] [PubMed]
73. Surette, M.E. The science behind dietary omega-3 fatty acids. *CMAJ* **2008**, *178*, 177–180. [CrossRef] [PubMed]
74. Cholewski, M.; Tomczykowa, M.; Tomczyk, M. A Comprehensive Review of Chemistry, Sources and Bioavailability of Omega-3 Fatty Acids. *Nutrients* **2018**, *10*, 1662. [CrossRef] [PubMed]
75. Calder, P.C.; Grimble, R.F. Polyunsaturated fatty acids, inflammation and immunity. *Eur. J. Clin. Nutr.* **2002**, *56* (Suppl. 3), S14–S19. [CrossRef] [PubMed]
76. SanGiovanni, J.P.; Chew, E.Y. The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina. *Prog. Retin. Eye Res.* **2004**, *24*, 87–138. [CrossRef] [PubMed]
77. Tuo, J.; Ross, R.J.; Herzlich, A.A.; Shen, D.; Ding, X.; Zhou, M.; Coon, S.L.; Hussein, N.; Salem, N., Jr.; Chan, C.-C. A High omega-3 fatty acid diet reduces retinal lesions in a murine model of macular degeneration. *Am. J. Pathol.* **2009**, *175*, 799–807. [CrossRef] [PubMed]
78. Calder, P.C. Omega-3 fatty acids and inflammatory processes: From molecules to man. *Biochem. Soc. Trans.* **2017**, *45*, 1105–1115. [CrossRef] [PubMed]
79. Gross, B.W.; Gillio, M.; Rinehart, C.D.; Lynch, C.A.; Rogers, F.B. Omega-3 Fatty Acid Supplementation and Warfarin: A Lethal Combination in Traumatic Brain Injury. *J. Trauma Nurs.* **2017**, *24*, 15–18. [CrossRef] [PubMed]
80. Prokopiou, E.; Kolovos, P.; Kalogerou, M.; Neokleous, A.; Papagregoriou, G.; Deltas, C.; Malas, S.; Georgiou, T. Therapeutic potential of omega-3 fatty acids supplementation in a mouse model of dry macular degeneration. *BMJ Open Ophthalmol.* **2017**, *1*, e000056. [CrossRef]
81. Dornstauder, B.; Suh, M.; Kuny, S.; Gaillard, F.; MacDonald, I.M.; Clandinin, M.T.; Sauvé, Y. Dietary docosahexaenoic acid supplementation prevents age-related functional losses and A2E accumulation in the retina. *Investig. Opthalmol. Vis. Sci.* **2012**, *53*, 2256–2265. [CrossRef] [PubMed]
82. Ramkumar, H.L.; Tuo, J.; Shen, D.F.; Zhang, J.; Cao, X.; Chew, E.Y.; Chan, C.C. Nutrient supplementation with n3 polyunsaturated fatty acids, lutein, and zeaxanthin decrease A2E accumulation and VEGF expression in the retinas of Ccl2/Cx3cr1-deficient mice on Crb1rd8 background. *J. Nutr.* **2013**, *143*, 1129–1135. [CrossRef] [PubMed]
83. Qiu, S.; Wei, Y.; Zhou, X.; Jiang, Z.; Zhang, T.; Jiang, X.; Zhang, S. Intravitreal injection of docosahexaenoic acid attenuated photoreceptor cell injury in a NaIO 3 -induced age-related macular degeneration rat model. *Neurosci. Lett.* **2017**, *657*, 53–61. [CrossRef] [PubMed]
84. Chew, E.Y.; Clemons, T.; SanGiovanni, J.P.; Danis, R.; Domalpally, A.; McBee, W.; Sperduto, R.; Ferris, F.L.; AREDS2 Research Group. The Age-Related Eye Disease Study 2 (AREDS2): Study design and baseline characteristics (AREDS2 report number 1). *Ophthalmology* **2012**, *119*, 2282–2289. [CrossRef] [PubMed]

85. Georgiou, T.; Prokopiou, E. The New Era of Omega-3 Fatty Acids Supplementation: Therapeutic Effects on Dry Age-Related Macular Degeneration. *J. Stem Cells* **2015**, *10*, 205–215. [PubMed]
86. Franzone, F.; Nebbioso, M.; Pergolizzi, T.; Attanasio, G.; Musacchio, A.; Greco, A.; Limoli, P.G.; Artico, M.; Spandidos, D.A.; Taurone, S.; et al. Anti-inflammatory role of curcumin in retinal disorders (Review). *Exp. Ther. Med.* **2021**, *22*, 790. [CrossRef]
87. Martínez-Solís, I.; Bosch-Morell, F.; Villagrasa, V.; Ortega, T.; Acero, N.; Muñoz-Mingarro, D.; González-Rosende, M.E.; Castillo, E.; Sanahuja, M.A.; Soriano, P. Medicinal plants and natural products as neuroprotective agents in age-related macular degeneration. *Neural Regen. Res.* **2020**, *15*, 2207–2216. [CrossRef] [PubMed]
88. Jurenka, J.S. Anti-inflammatory properties of curcumin, a major constituent of Curcuma longa: A review of preclinical and clinical research. *Altern. Med. Rev.* **2009**, *14*, 141–153. [PubMed]
89. Aggarwal, B.B.; Harikumar, K.B. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. *Int. J. Biochem. Cell Biol.* **2008**, *41*, 40–59. [CrossRef]
90. Schaffer, M.; Schaffer, P.M.; Zidan, J.; Sela, G.B. Curcuma as a functional food in the control of cancer and inflammation. *Curr. Opin. Clin. Nutr. Metab. Care* **2011**, *14*, 588–597. [CrossRef]
91. Hewlings, S.J.; Kalman, D.S. Curcumin: A Review of Its Effects on Human Health. *Foods* **2017**, *6*, 92. [CrossRef] [PubMed]
92. Allegra, A.; Innao, V.; Russo, S.; Gerace, D.; Alonci, A.; Musolino, C. Anticancer Activity of Curcumin and Its Analogues: Preclinical and Clinical Studies. *Cancer Investig.* **2017**, *35*, 1–22. [CrossRef] [PubMed]
93. Radomska-Leśniewska, D.M.; Osiecka-Iwan, A.; Hyc, A.; Góźdź, A.; Dąbrowska, A.M.; Skopiński, P. Therapeutic potential of curcumin in eye diseases. *Cent. Eur. J. Immunol.* **2019**, *44*, 181–189. [CrossRef] [PubMed]
94. Peddada, K.V.; Brown, A.; Verma, V.; Nebbioso, M. Therapeutic potential of curcumin in major retinal pathologies. *Int. Ophthalmol.* **2018**, *39*, 725–734. [CrossRef] [PubMed]
95. Mandal, N.A.; Patlolla, J.M.; Zheng, L.; Agbaga, M.-P.; Tran, J.-T.A.; Wicker, L.; Kasus-Jacobi, A.; Elliott, M.H.; Rao, C.V.; Anderson, R.E. Curcumin protects retinal cells from light-and oxidant stress-induced cell death. *Free Radic. Biol. Med.* **2009**, *46*, 672–679. [CrossRef] [PubMed]
96. Lu, J.; Zhu, W.; Wu, Y.; Meng, Y.-F.; Wang, J.-Y.; Xu, M.; Tao, J.-J. Effect of curcumin on aging retinal pigment epithelial cells. *Drug Des. Dev. Ther.* **2015**, *9*, 5337–5344. [CrossRef] [PubMed]
97. Howell, J.C.; Chun, E.; Farrell, A.N.; Hur, E.Y.; Caroti, C.M.; Iuvone, P.M.; Haque, R. Global microRNA expression profiling: Curcumin (diferuloylmethane) alters oxidative stress-responsive microRNAs in human ARPE-19 cells. *Mol. Vis.* **2013**, *19*, 544–560. [PubMed]
98. Saberi-Karimian, M.; Katsiki, N.; Caraglia, M.; Boccellino, M.; Majeed, M.; Sahebkar, A. Vascular endothelial growth factor: An important molecular target of curcumin. *Crit. Rev. Food Sci. Nutr.* **2019**, *59*, 299–312. [CrossRef] [PubMed]
99. Anand, P.; Kunnumakkara, A.B.; Newman, R.A.; Aggarwal, B.B. Bioavailability of curcumin: Problems and promises. *Mol. Pharm.* **2007**, *4*, 807–818. [CrossRef]
100. Pescosolido, N.; Giannotti, R.; Plateroti, A.M.; Pascarella, A.; Nebbioso, M. Curcumin: Therapeutical potential in ophthalmology. *Planta Medica* **2013**, *80*, 249–254. [CrossRef]
101. Soleimani, V.; Sahebkar, A.; Hosseinzadeh, H. Turmeric (Curcuma longa) and its major constituent (curcumin) as nontoxic and safe substances: Review. *Phyther. Res.* **2018**, *32*, 985–995. [CrossRef] [PubMed]
102. Heitmar, R.; Brown, J.; Kyrou, I. Saffron (*Crocus sativus* L.) in Ocular Diseases: A Narrative Review of the Existing Evidence from Clinical Studies. *Nutrients* **2019**, *11*, 649. [CrossRef] [PubMed]
103. Hatziagapiou, K.; Kakouri, E.; Lambrou, G.I.; Bethanis, K.; Tarantilis, P.A. Antioxidant Properties of *Crocus sativus* L. and Its Constituents and Relevance to Neurodegenerative Diseases; Focus on Alzheimer's and Parkinson's Disease. *Curr. Neuropharmacol.* **2019**, *17*, 377–402. [CrossRef] [PubMed]
104. Di Marco, S.; Carnicelli, V.; Franceschini, N.; Di Paolo, M.; Piccardi, M.; Bisti, S.; Falsini, B. Saffron: A Multitask Neuroprotective Agent for Retinal Degenerative Diseases. *Antioxidants* **2019**, *8*, 224. [CrossRef] [PubMed]
105. Nam, K.N.; Park, Y.-M.; Jung, H.-J.; Lee, J.Y.; Min, B.D.; Park, S.-U.; Jung, W.-S.; Cho, K.-H.; Park, J.-H.; Kang, I.; et al. Anti-inflammatory effects of crocin and crocetin in rat brain microglial cells. *Eur. J. Pharmacol.* **2010**, *648*, 110–116. [CrossRef] [PubMed]
106. Ohno, Y.; Nakanishi, T.; Umigai, N.; Tsuruma, K.; Shimazawa, M.; Hara, H. Oral administration of crocetin prevents inner retinal damage induced by N-methyl-d-aspartate in mice. *Eur. J. Pharmacol.* **2012**, *690*, 84–89. [CrossRef]
107. Xuan, B.; Zhou, Y.-H.; Li, N.; Min, Z.-D.; Chiou, G.C. Effects of crocin analogs on ocular blood flow and retinal function. *J. Ocul. Pharmacol. Ther.* **1999**, *15*, 143–152. [CrossRef] [PubMed]
108. Falsini, B.; Piccardi, M.; Minnella, A.; Savastano, C.; Capoluongo, E.; Fadda, A.; Balestrazzi, E.; Maccarone, R.; Bisti, S. Influence of saffron supplementation on retinal flicker sensitivity in early age-related macular degeneration. *Investig. Ophthalmol. Vis. Sci.* **2010**, *51*, 6118–6124. [CrossRef]
109. Piccardi, M.; Marangoni, D.; Minnella, A.M.; Savastano, M.C.; Valentini, P.; Ambrosio, L.; Capoluongo, E.; Maccarone, R.; Bisti, S.; Falsini, B. A Longitudinal follow-up study of saffron supplementation in early age-related macular degeneration: Sustained benefits to central retinal function. *Evid.-Based Complement. Altern. Med.* **2012**, *2012*, 429124. [CrossRef]

110. Marangoni, D.; Falsini, B.; Piccardi, M.; Ambrosio, L.; Minnella, A.M.; Savastano, M.C.; Bisti, S.; Maccarone, R.; Fadda, A.; Mello, E.; et al. Functional effect of Saffron supplementation and risk genotypes in early age-related macular degeneration: A preliminary report. *J. Transl. Med.* **2013**, *11*, 228. [CrossRef]
111. Lashay, A.; Sadough, G.; Ashrafi, E.; Lashay, M.; Movassat, M.; Akhondzadeh, S. Short-term Outcomes of Saffron Supplementation in Patients with Age-related Macular Degeneration: A Double-blind, Placebo-controlled, Randomized Trial. *Med. Hypothesis Discov. Innov. Ophthalmol.* **2016**, *5*, 32–38.
112. Riazi, A.; Panahi, Y.; Alishiri, A.A.; Hosseini, M.A.; Karimi Zarchi, A.A.; Sahebkar, A. The impact of saffron (*Crocus sativus*) supplementation on visual function in patients with dry age-related macular degeneration. *Ital. J. Med.* **2016**, *10*, 196–201. [CrossRef]
113. Broadhead, G.K.; Grigg, J.R.; McCluskey, P.; Hong, T.; Schlub, T.E.; Chang, A.A. Saffron therapy for the treatment of mild/moderate age-related macular degeneration: A randomised clinical trial. *Graefes Arch. Clin. Exp. Ophthalmol.* **2019**, *257*, 31–40. [CrossRef]
114. Amaral, F.G.D.; Cipolla-Neto, J. A brief review about melatonin, a pineal hormone. *Arq. Bras. de Endocrinol. Metabol.* **2018**, *62*, 472–479. [CrossRef] [PubMed]
115. Alarma-Estrany, P.; Pintor, J. Melatonin receptors in the eye: Location, second messengers and role in ocular physiology. *Pharmacol. Ther.* **2006**, *113*, 507–522. [CrossRef]
116. Alkozi, H.A.; Wang, X.; de Lara, M.J.P.; Pintor, J. Presence of melanopsin in human crystalline lens epithelial cells and its role in melatonin synthesis. *Exp. Eye Res.* **2017**, *154*, 168–176. [CrossRef]
117. Reiter, R.J.; Tan, D.X.; Fuentes-Broto, L. Melatonin: A multitasking molecule. *Prog Brain Res.* **2010**, *181*, 127–151.
118. Poeggeler, B.; Reiter, R.J.; Tan, D.X.; Chen, L.D.; Manchester, L.C. Melatonin, hydroxyl radical-mediated oxidative damage, and aging: A hypothesis. *J. Pineal Res.* **1993**, *14*, 151–168. [CrossRef]
119. Crooke, A.; Huete-Toral, F.; Colligris, B.; Pintor, J. The role and therapeutic potential of melatonin in age-related ocular diseases. *J. Pineal Res.* **2017**, *63*, e12430. [CrossRef] [PubMed]
120. Dehdashtian, E.; Mehrzadi, S.; Yousefi, B.; Hosseinzadeh, A.; Reiter, R.J.; Safa, M.; Ghaznavi, H.; Naseripour, M. Diabetic retinopathy pathogenesis and the ameliorating effects of melatonin; involvement of autophagy, inflammation and oxidative stress. *Life Sci.* **2018**, *193*, 20–33. [CrossRef]
121. Mehrzadi, S.; Hemati, K.; Reiter, R.J.; Hosseinzadeh, A. Mitochondrial dysfunction in age-related macular degeneration: Melatonin as a potential treatment. *Expert Opin. Ther. Targets* **2020**, *24*, 359–378. [CrossRef] [PubMed]
122. Fu, Y.; Tang, M.; Fan, Y.; Zou, H.; Sun, X.; Xu, X. Anti-apoptotic effects of melatonin in retinal pigment epithelial cells. *Front. Biosci.* **2012**, *17*, 1461–1468. [CrossRef] [PubMed]
123. Rosen, R.; Hu, D.-N.; Perez, V.; Tai, K.; Yu, G.-P.; Chen, M.; Tone, P.; McCormick, S.A.; Walsh, J. Urinary 6-sulfatoxymelatonin level in age-related macular degeneration patients. *Mol. Vis.* **2009**, *15*, 1673–1679. [PubMed]
124. Yi, C.; Pan, X.; Yan, H.; Guo, M.; Pierpaoli, W. Effects of melatonin in age-related macular degeneration. *Ann. N. Y. Acad. Sci.* **2005**, *1057*, 384–392. [CrossRef] [PubMed]
125. Yao, J.; Jia, L.; Shelby, S.J.; Ganios, A.M.; Feathers, K.; Thompson, D.A.; Zacks, D.N. Circadian and noncircadian modulation of autophagy in photoreceptors and retinal pigment epithelium. *Investig. Opthalmol. Vis. Sci.* **2014**, *55*, 3237–3246. [CrossRef] [PubMed]
126. Grace, M.S.; Chiba, A.; Menaker, M. Circadian control of photoreceptor outer segment membrane turnover in mice genetically incapable of melatonin synthesis. *Vis. Neurosci.* **1999**, *16*, 909–918. [CrossRef] [PubMed]
127. Organisciak, D.T.; Darrow, R.M.; Barsalou, L.; Kutty, R.K.; Wiggert, B. Circadian-dependent retinal light damage in rats. *Investig. Ophthalmol. Vis. Sci.* **2000**, *41*, 3694–3701.
128. Seagle, B.-L.L.; Rezai, K.A.; Kobori, Y.; Gasyna, E.M.; Rezaei, K.A.; Norris, J.R. Melanin photoprotection in the human retinal pigment epithelium and its correlation with light-induced cell apoptosis. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 8978–8983. [CrossRef] [PubMed]
129. Kaur, C.; Sivakumar, V.; Yong, Z.; Lu, J.; Foulds, W.; Ling, E. Blood–retinal barrier disruption and ultrastructural changes in the hypoxic retina in adult rats: The beneficial effect of melatonin administration. *J. Pathol.* **2007**, *212*, 429–439. [CrossRef]
130. Ambati, J.; Ambati, B.K.; Yoo, S.H.; Ianchulev, S.; Adamis, A.P. Age-related macular degeneration: Etiology, pathogenesis, and therapeutic strategies. *Surv. Ophthalmol.* **2003**, *48*, 257–293. [CrossRef]
131. Pameijer, E.M.; Heus, P.; Damen, J.A.A.; Spijker, R.; Hooft, L.; Ringens, P.J.; Imhof, S.M.; van Leeuwen, R. What did we learn in 35 years of research on nutrition and supplements for age-related macular degeneration: A systematic review. *Acta Ophthalmol.* **2022**, *100*, E1541–E1552. [CrossRef] [PubMed]
132. Wu, Y.; Xie, Y.; Yuan, Y.; Xiong, R.; Hu, Y.; Ning, K.; Ha, J.; Wang, W.; Han, X.; He, M. The Mediterranean Diet and Age-Related Eye Diseases: A Systematic Review. *Nutrients* **2023**, *15*, 2043. [CrossRef] [PubMed]
133. Merle, B.M.; Silver, R.E.; Rosner, B.; Seddon, J.M. Adherence to a Mediterranean diet, genetic susceptibility, and progression to advanced macular degeneration: A prospective cohort study. *Am. J. Clin. Nutr.* **2015**, *102*, 1196–1206. [CrossRef]
134. Merle, B.M.; Colijn, J.M.; Cougnard-Grégoire, A.; de Koning-Backus, A.P.; Delyfer, M.N.; Kiefte-de Jong, J.C.; Meester-Smoor, M.; Féart, C.; Verzijden, T.; Samieri, C.; et al. Mediterranean Diet and Incidence of Advanced Age-Related Macular Degeneration: The EYE-RISK Consortium. *Ophthalmology* **2019**, *126*, 381–390. [CrossRef] [PubMed]

135. Chapman, N.A.; Jacobs, R.J.; Braakhuis, A.J. Role of diet and food intake in age-related macular degeneration: A systematic review. *Clin. Exp. Ophthalmol.* **2018**, *47*, 106–127. [CrossRef] [PubMed]
136. Chong, E.W.; Kreis, A.J.; Wong, T.Y.; Simpson, J.A.; Guymer, R.H. Dietary omega-3 fatty acid and fish intake in the primary prevention of age-related macular degeneration: A systematic review and meta-analysis. *Arch. Ophthalmol.* **2008**, *126*, 826–833. [CrossRef] [PubMed]
137. Christen, W.G.; Schaumberg, D.A.; Glynn, R.J.; Buring, J.E. Dietary omega-3 fatty acid and fish intake and incident age-related macular degeneration in women. *Arch. Ophthalmol.* **2011**, *129*, 921–929. [CrossRef] [PubMed]
138. Chong, E.W.-T.; Simpson, J.A.; Robman, L.D.; Hodge, A.M.; Aung, K.Z.; English, D.R.; Giles, G.G.; Guymer, R.H. Red meat and chicken consumption and its association with age-related macular degeneration. *Am. J. Epidemiol.* **2009**, *169*, 867–876. [CrossRef] [PubMed]
139. Kaushik, S.; Wang, J.J.; Flood, V.; Tan, J.S.L.; Barclay, A.W.; Wong, T.Y.; Brand-Miller, J.; Mitchell, P. Dietary glycemic index and the risk of age-related macular degeneration. *Am. J. Clin. Nutr.* **2008**, *88*, 1104–1110. [CrossRef]
140. Chiu, C.-J.; Milton, R.C.; Klein, R.; Gensler, G.; Taylor, A. Dietary carbohydrate and the progression of age-related macular degeneration: A prospective study from the Age-Related Eye Disease Study. *Am. J. Clin. Nutr.* **2007**, *86*, 1210–1218. [CrossRef]
141. Dinu, M.; Pagliai, G.; Casini, A.; Sofi, F. Food groups and risk of age-related macular degeneration: A systematic review with meta-analysis. *Eur. J. Nutr.* **2019**, *58*, 2123–2143. [CrossRef] [PubMed]
142. Kuan, V.; Warwick, A.; Hingorani, A.; Tufail, A.; Cipriani, V.; Burgess, S.; Sofat, R.; Fritsche, L.G.; Igl, W.; Bailey, J.N.; et al. Association of Smoking, Alcohol Consumption, Blood Pressure, Body Mass Index, and Glycemic Risk Factors With Age-Related Macular Degeneration: A Mendelian Randomization Study. *JAMA Ophthalmol.* **2021**, *139*, 1299–1306. [CrossRef] [PubMed]
143. Lixi, F.; Vitiello, L.; Giannaccare, G. Marine Natural Products Rescuing the Eye: A Narrative Review. *Mar. Drugs* **2024**, *22*, 155. [CrossRef] [PubMed]
144. Vitiello, L.; Capasso, L.; Cembalo, G.; De Pascale, I.; Imparato, R.; De Bernardo, M. Herbal and Natural Treatments for the Management of the Glaucoma: An Update. *BioMed Res. Int.* **2023**, *2023*, 3105251. [CrossRef]
145. D'angelo, A.; Vitiello, L.; Lixi, F.; Abbinante, G.; Coppola, A.; Gagliardi, V.; Pellegrino, A.; Giannaccare, G. Optic Nerve Neuroprotection in Glaucoma: A Narrative Review. *J. Clin. Med.* **2024**, *13*, 2214. [CrossRef]

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