Clinical Ophthalmology

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Color Doppler Imaging, Endothelin-1, Corneal Biomechanics and Scleral Rigidity in Asymmetric Age-Related Macular Degeneration

Alessandro Finzi[1], Simone Ottoboni[1], Mauro Cellini[1], Beniamino Corcioni[2], Caterina Gaudiano[2], Luigi Fontana[1]

[1]Ophthalmology Unit, Department of Experimental, Diagnostic and Specialty Medicine, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy; [2]Department of Radiology, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy

Correspondence: Alessandro Finzi, Ophthalmology Unit, Department of Experimental, Diagnostic and Specialty Medicine, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Via Pelagio Palagi 9, Bologna, 40138, Italy, Tel +39 051 2142831, Email finzialessandro@gmail.com

Purpose: Age-related macular degeneration (AMD) presents a multifaceted etiopathogenesis involving ischemic, inflammatory, and genetic components. This study investigates the correlation between ocular hemodynamics, scleral rigidity (SR), and plasma endothelin-1 (ET1) levels in treatment-naive patients with asymmetrical AMD.

Patients and Methods: This study included 20 treatment-naive patients (12 females and 8 males) with an average age of 76.4 ± 3.7 years, who presented with AMD with neovascular membrane formation (nAMD) in one eye, and intermediate grade 2 AMD (iAMD) in the other eye. The control group consisted of 20 healthy subjects (13 females and 7 males) with a mean age of 74.7 ± 3.9 years. All patients and healthy controls underwent color Doppler imaging (i) of the ophthalmic artery (OA), short posterior ciliary arteries (SPCAs), and central retinal artery (CRA); Plasma ET-1 levels were measured for all patients and healthy subjects. Corneal biomechanics were assessed using an Ocular Response Analyzer and two indices were obtained: corneal hysteresis (CH) and corneal resistance factor (CRF).

Results: Results showed reduced blood flow velocities and increased resistance indices in AMD eyes, particularly affecting the short posterior ciliary arteries. According to mechanical theory, ARMD eyes exhibited elevated scleral rigidity and corneal resistance factor compared to controls, with a notable rise in SR in neovascular AMD (nAMD) eyes. As per the chronic subacute inflammation theory, plasma ET-1 levels were significantly higher in AMD patients, correlating with abnormal SPCAs blood flow and increased resistance indices.

Conclusion: Findings suggest a multifactorial etiology of AMD involving an increase of ET-1 plasma levels with biomechanic damages of corneal and scleral tissue in nAMD.

Keywords: AMD, endothelin-1, corneal biomechanics, scleral rigidity, hemodynamics

Introduction

The etiopathogenesis of age-related macular degeneration (AMD) is particularly complex, involving oxidative, ischemic, inflammatory, and genetic factors.[1–3] An important aspect is the choroidal ischemic component, which can lead to the onset of both types of AMD, the dry AMD and the wet AMD (with neo angiogenesis). Two principal hypotheses have been proposed to explain the onset of choroidal ischemia.[4]

The first theory suggest that the ischemia is due to the production of substances with angiogenic properties that not only damage the cardiovascular system but also affect the choriocapillaris, Bruch's membrane, and the retinal pigment epithelium (RPE).[5] Endothelin-1 (ET-1) is the most potent and long-acting vasoconstrictor peptide with binding sites in many ocular tissues such as iris, ciliary processes, RPE and both choroidal and retinal vasculature. Several studies have found increased ET-1 plasma levels in various ocular diseases.[6,7] A second hypothesis posits that over time, there is

Received: 21 May 2024
Accepted: 2 September 2024
Published: 10 September 2024

a progressive increase in scleral stiffness, leading to venous blood pooling within the rigid structure; according to Starling's law, this results in a slowdown of choriocapillaris circulation and subsequent focal ischemia.[8]

Based on these assumptions, we aimed to evaluate a possible correlation between ocular hemodynamic data of treatment-naive patients with asymmetrical AMD, and their scleral rigidity (SR) values and plasma endothelin-1 (ET-1) levels.

Materials and Methods

The study was conducted in accordance with the Declaration of Helsinki and informed consent was obtained from all subjects.

This study included 20 treatment-naive patients (12 females and 8 males) with an average age of 76.4 ± 3.7 years, who presented with AMD with neovascular membrane formation (nAMD) in one eye, and intermediate grade 2 AMD (iAMD) in the other eye.[9] The AMD was diagnosed by Optical Coherence Tomography (OCT), Fluorangiography (FA) and Indocyanine Green Angiography (ICGA) (Heidelberg Spectralis, Heidelberg Engineering, Heidelberg, Germany).

The control group consisted of 20 healthy subjects (13 females and 7 males) with a mean age of 74.7 ± 3.9 years (Table 1).

The exclusion criteria included regular tobacco use and systemic diseases that could interfere with endothelial function, such as hypertension, heart failure, diabetes mellitus, and dyslipidemia; in addition, patients with previous cerebrovascular problems, autoimmune diseases, and those taking vasoactive drugs were excluded.

Plasma ET-1 levels were measured for all patients and healthy subjects in the control group. Venous blood samples were obtained from an antecubital vein and placed in a refrigerated container with EDTA and ice. The blood was then centrifuged at 4°C, frozen at −25°C, and ET-1 extraction was performed using a C-18 Sep-column pack (Peninsula Laboratories, Belmont, CA, USA). The concentration of ET-1 (picograms/milliliter) was subsequently determined using a commercial radioimmunoassay (ELISA) kit (T-4050 BMA Biomedicals, Peninsula Laboratories, Belmont, CA, USA).[7]

All patients and healthy controls underwent color Doppler imaging (CDI) of the ophthalmic artery (OA), short posterior ciliary arteries (SPCAs), and central retinal artery (CRA) using an Aplio 500 ultrasound machine (Toshiba Medical System, Tokyo, Japan) with a 7.5 MHz linear probe, following established protocols.[10–12] The examinations were conducted by an experienced sonographer in a blinded manner. In short, the ultrasound probe was placed on the closed eyelid of the eye being examined with the patient in a supine position and positioned to optimize the signal from the vessel being examined. The OA was examined in one of three segments: laterally, from above, or medially of the optic nerve shadow. The CRA was examined 2 mm anterior to the shadow of the optic nerve, and the SPCAs were visualized on the temporal or nasal side of the optic nerve shadow, approximately 10–15 mm behind the ultrasonographic image of the globe.

The ultrasound software recorded three main parameters: peak systolic velocity (PSV), end diastolic velocity (EDV) and resistance index (RI); this last parameter is related to the resistance present downstream of the sampling point of the vessel examined. Therefore, these parameters allow the assessment of any circulatory damage.[13]

SR values were determined using the double weighing method with a Schiötz tonometer, utilizing 5.5 g and 10.0 g weights; the reading of the two weights on the tonometric scale were applied to the Friedenwald nomogram to obtain the values of SR.[14]

Table 1 Demographic Data of the Participants to the Study

	AMD	Control Group	$^{§}p<0.05$
Age, years	76.4±3.7	74.7±3.9	0.1619
CI 95%	74.6–78.1	72.8–76.5	
Gender, M:F	8:12	7:13	
Ethnicity	100% Caucasian	100% Caucasian	

Notes: §Mann–Whitney *U*-test.
Abbreviations: F, female; M, male; AMD, age-related macular degeneration; CI, confidence interval.

Corneal biomechanics were assessed using an Ocular Response Analyzer (ORA Reichert Ophthalmic Instruments Inc, Depew, NY, USA). The instrument has an integrated infrared beam that records the flattening of the cornea during the inward and outward corneal response to an air pulse lasting approximately 20 milliseconds. The collected corneal displacement data allow for the calculation of two indices: corneal hysteresis (CH) and corneal resistance factor (CRF).

CRF has been shown to be relatively independent of intraocular pressure (IOP) compared to CH.[15–17] Recent experimental studies, both ex-vivo and using finite-element eye modeling, have highlighted the relationship between corneal biomechanics, particularly CRF, and scleral biomechanics.[17–19]

For the statistical analysis we used the MedCalc 10.9.1 statistical software (MedCalc Software, Ostend, Belgium). The data underwent analysis with the Mann–Whitney *U*-test to compare differences between groups, Wilcoxon's signed-rank test for within-subject comparisons, and Spearman correlation test, with statistical significance set at $p < 0.05$.

Results

The CDI examination revealed a general reduction in PSV in patients with AMD compared to the control group, affecting all examined vessels in both eyes with nAMD and iAMD, with moderate statistical variability depending on the vessel examined (Table 2). PSV was significantly reduced in the eye affected by nAMD ($p = 0.0063$) at both SPCAs ($p = 0.0056$) and the CRA ($p = 0.0453$) compared to the healthy group. RI showed a marked increase in all examined vessels in AMD eyes compared to the control group. Moreover, RI showed a significant increase in nAMD eyes compared to iAMD eyes ($p = 0.0363$).

Table 2 Summary Table of the Color Doppler Flowmetry Survey

	nAMD	iAMD	*p<0.05	Control Group (CG)	§p<0.05 CG vs nAMD	§p<0.05 CG vs iAMD
PSV OA	48.40 ± 8.03	48.43 ± 8.86	0.995	48.56 ± 10.98	0.602	0.678
CI 95%	44.46–52.16	44.28–52.57		43.43–53.71		
EDV OA	8.30 ± 1.88	10.46 ± 2.86	0.0138	9.48 ± 2.91	0.088	0.429
CI 95%	7.42–9.18	9.12–11.80		8.2–10.85		
RI OA	0.83 ± 0.03	0.83 ± 0.05	0.684	0.76 ± 0.04	0.0001	0.0001
CI 95%	0.82–0.84	0.74–0.78		0.81–0.86		
PSV SPCAs	12.37 ± 2.80	13.36 ± 3.60	0.0764	15.44 ± 3.59	0.0056	0.0559
CI 95%	11.06–13.68	11.68–15.05		13.76–17.12		
EDV SPCAs	2.74 ± 0.89	3.61 ± 1.41	0.0001	3.98 ± 0.93	0.0214	0.4017
CI 95%	2.69–2.80	2.95–4.27		3.54–4.42		
RI SPCAs	0.80 ± 0.03	0.77 ± 0.07	0.0363	0.74 ± 0.02	0.0001	0.0305
CI 95%	0.79–0.81	0.74–0.81		0.73–0.75		
PSV CRA	13.28 ± 1.80	13.88 ± 2.77	0.0385	14.15 ± 1.49	0.0453	0.695
CI 95%	12.43–14.12	12.59–15.18		13.45–14.85		
EDV CRA	2.79 ± 0.80	3.30 ± 0.98	0.0483	3.14 ± 0.63	0.133	0.543
CI 95%	2.42–3.15	2.84–3.76		2.85–3.44		
RI CRA	0.80 ± 0.03	0.82 ± 0.03	0.5763	0.76 ± 0.03	0.0067	0.0424
CI 95%	0.78–0.80	0.77–0.79		0.75–0.77		

Notes: *Wilcoxon signed-rank test; §Mann–Whitney *U*-test.

Abbreviations: OA, Ophthalmic Artery; SPCAs, Posterior Short Ciliary Arteries; CRA, Central Retinal Artery; nAMD, neovascular age-related macular degeneration; iAMD, intermediate age-related macular degeneration; PSV, peak systolic velocity; EDV, end diastolic velocity; RI, resistance index; CG, control group; CI, confidence interval).

CH and CRF values found in AMD eyes and in healthy controls are summarized in Table 3. Eyes affected by nAMD showed increased CH compared to healthy controls (p = 0.0106) and iAMD (p = 0.0186). Furthermore, CRF was higher in nAMD compared to both healthy controls (p = 0.0001) and iAMD (p = 0.0256), which, in turn, exhibited an increase in CRF compared to the control group (p = 0.0025). SR values determined using the Friedenwald nomogram demonstrated a statistically significant increase in eyes affected by both nAMD (p = 0.0004) and iAMD (p = 0.0077) compared to healthy controls; nAMD eyes had significantly higher SR than iAMD eyes (p = 0.0033).

In AMD patients, there was a significant increase in ET-1 plasma levels compared to the control group (p = 0.0006) (Table 3).

At the SPCAs level, in both eyes with nAMD and fellow eyes with iAMD, an increase in ET-1 values correlated with a reduction in PSV (nAMD: p = 0.001; iAMD: p = 0.006) and an increase in RI (nAMD: p = 0.001; iAMD: p = 0.001) (Table 4).

Additionally, the significant increase in RI in the SPCAs of nAMD eyes compared to fellow iAMD eyes (p = 0.0363) correlated with scleral stiffness, showing an increase in CRF (r = 0.52; p < 0.020) and SR (r = 0.48; p = 0.033) (Table 5).

Table 3 Correlation Between Mechanical Factors and AMD

	nAMD	iAMD	*p<0.05	Control Group (CG)	§p<0.05 CG vs nAMD	§p<0.05 CG vs iAMD
CH (mm/Hg)	11.18 ± 1.06	10.15 ± 1.25	0.0186	10.02 ±1.70	0.0106	0.6167
CI 95%	10.69–11.70	9.57–10.74		9.24–10.81		
CRF (mm/Hg)	12.24 ± 1.05	11.23 ±1.30	0.0256	9.82 ± 1.36	0.0001	0.0025
CI 95%	11.77–12.71	10.53–11.74		9.18–10.46		
CCT (µm)	530 ± 11	528 ± 12	0.7937	526 ± 12	0.2612	0.5516
CI 95%	525–535	522–534		520–532		
SR	0.0385 ± 0.005	0.0358 ± 0.004	0.0033	0.0328 ± 0.004	0.0001	0.0077
CI 95%	0.0363–0.0408	0.0340–0.0375		0.031–0.0346		
ET-1 (pg/mL)	2.060 ± 0.276	2.060 ± 0.276	–	1.492 ± 0.0.560	0.0006	0.0006
CI 95%	1.931–2.188	1.931–2.188		1.225–1.758		

Notes: *Wilcoxon signed-rank test; §Mann–Whitney *U*-test.

Abbreviations: nAMD, neovascular age-related macular degeneration; iAMD, intermediate age-related macular degeneration; CH, corneal hysteresis; CRF, corneal resistance factor; SR, scleral rigidity; ET-1, endothelin-1; CG, control group; CCT, corneal central thickness; CI, Confidence interval.

Table 4 Spearman Correlation Test Between ET-1 and PSV and RI Values in SPCAs of Patients with Asymmetric AMD

nAMD	SPCAs-PSV	SPCAs-RI	iAMD	SPCAs-PSV	SPCA-RI
ET-1	r = 0.70	r = 0.78	ET-1	r = 0.59	r = 0.88
	p<0.001	p<0.001		p<0.006	p<0.001

Abbreviations: ET-1, endothelin-1; nAMD, neovascular age-related macular degeneration; iAMD, intermediate age-related macular degeneration; SPCAs, Posterior Short Ciliary Arteries; PSV, peak systolic velocity; RI, resistance index.

Table 5 Spearman Correlation Test Between CRF and SR with PSV and RI Values in SPCAs of Patients with Asymmetric AMD

nAMD	PSV-SPCAs	RI-SPCAs	iAMD	PSV-SPCAs	RI-SPCAs
CRF	0.347	**r=0.520**	CRF	−0.145	0.144
	p<0.133	**p<0.020**		p<0.543	p<0.544
SR	−0.179	**r=0.480**	SR	−0.104	−0.020
	p<0.451	**p<0.033**		p<0.663	p<0.935

Note: Statistically significant correlation values are in bold.
Abbreviations: CRF, Corneal Resistance Factor; SR, Scleral Rigidity; PSV, Peak Systolic Velocity; RI, Resistance Index; SPCAs, Short Posterior Ciliary Arteries; nAMD, neovascular age-related macular degeneration; iAMD, Intermediate age-related macular degeneration.

Discussion

The objective of our study was to analyze the blood flow of the orbital vessels using color Doppler imaging in a cohort of patients with asymmetrical age-related macular degeneration, with the aim of exploring potential correlations between flowmetric indices, scleral rigidity values, and plasma endothelin-1 levels. Our study revealed that, compared to the healthy group, in naive patients with asymmetric AMD there is a significant increase of RI in all vessels examined both in nAMD and iAMD particularly affecting the SPCAs, with notable differences between nAMD and iAMD eyes. Furthermore, we observed a notable rise in SR and CRF in both eyes of AMD patients compared to controls, with a particularly high increase in SR in nAMD eyes compared to their fellow eyes; our results demonstrated a correlation between SR values and CRF, supporting the results found and validated by previous articles.[20,21]

Additionally, plasma ET-1 levels were markedly elevated in AMD patients compared to the control group, with a correlation observed between ET-1 values, abnormal SPCAs blood flow rates, and increased RI in both nAMD and iAMD eyes.

Previous studies on patients with asymmetrical AMD have shown mixed results. For example, Sandhu et al[22] found no significant differences in pulsatile ocular blood flow (POBF) between the two eyes of Caucasian patients with asymmetrical AMD.

In contrast, earlier Chinese studies by Chen et al[23,24] reported a significant reduction in blood flow in the eye affected by choroidal neovascular membrane (CNVM) compared to the contralateral eye during the active and scarring phases.

We believe that these varying outcomes may be attributed to differences in the assessment techniques employed; indeed, POBF assessment evaluates overall choroidal blood flow and may not detect subtle ischemic areas at the choriocapillaris level and could be influenced by variations in scleral stiffness.[25] Our investigation using the CDI technique confirms the findings of both Chen et al and Boltz et al.[24,26]

Our flowmetric data align with the findings of previous investigations utilizing various methods such as POBF studies and laser Doppler flowmetry (LDF) studies.[27–32] These studies collectively suggest that choroidal ischemia plays a key role in the development of AMD in both its dry and wet forms.[30,33–35]

The etiopathogenesis of choroidal ischemia in AMD has been explored through various theories. A mechanical theory proposed by Friedman[36] hypothesizes that, with the aging processes of the ocular tissues, there is an increase in scleral stiffness leading to an increase in resistance to venous outflow and, therefore, according to Starling's law, a consequent reduction of choriocapillaris perfusion.[8]

Our study of SR detected using the Friedenwald nomogram[14] corroborates these findings observed by Friedman and Pallikaris et al.[37] Furthermore, at the level of SPCAs, the increase in RI in eyes with nAMD has a significant correlation with the increase in SR and CRF. This correlation confirms the results found and validated by previous articles;[20,21] these data would also confirm that the CRF index is a reliable expression of the scleral stiffness as hypothesized by us[19] and demonstrated by recent studies on ocular biomechanics.[17,18] However, these results seem to confirm that choroidal ischemia appears to have a multifactorial, and not purely mechanical, etiology.

The factors involved may be genetic,[38,39] metabolic[40,41] or related to the tissue aging processes.[42] Chronic subclinical inflammation emerges as a common underlying factor that can contribute to and sustain retinal damage.[43]

Genetic factors, particularly those related to chromosome 1[44] and inflammation-regulating genes like complement factor H (CFH), are significantly associated with AMD.[45,46] CFH is a circulating protein that both directly and indirectly inhibits the three complement activation pathways;[44] the presence of complement factors within drusen[47–49] would therefore lead to the hypothesis that AMD may derive from an aberrant inflammatory reaction caused by a downregulation of the complement system.[50]

Several studies have explored genetic variants, especially the CFH variant Y402H, in ARMD development and progression, albeit with conflicting results.[51–53]

Metabolic factors contributing to the development of AMD are closely associated with retinal aging processes and involve various mechanisms; these factors include the formation of drusen containing pro-inflammatory molecules,[54,55] thickening and stiffening of Bruch's membrane due to lipid accumulation,[44] and damage to the RPE.

Aging of RPE cells leads to metabolic alterations in the digestion process of photoreceptor outer segments, resulting in the accumulation of lipofuscin.[40,56] Additionally, oxidative processes determine an increase in chromophore A2E,[41,57] capable of activating not only the complement cascade through the onset of a chronic subacute inflammatory reaction,[41,58] but also the NLRP3 inflammasome.[40,41,59–61] The NLRP3 inflammasome activation may lead to abnormal production of vasoactive molecules by RPE cells, further exacerbating the inflammatory response and potentially contributing to the pathogenesis of AMD.[62]

The role of inflammation in AMD, particularly involving choriocapillaris cells, has been highlighted by studies showing an increase in plasma inflammatory markers such as C-reactive protein and IL-6. Additionally, markers associated with endothelial dysfunction, including systemic and choroidal soluble vascular cell adhesion molecule-1 (sVCAM)[54,55,63] were found to be elevated, along with an increase in plasma homocysteine levels.[64,65] The presence of diffuse endothelial dysfunction in AMD was confirmed using the flow mediated dilation (FMD) test, which is non-invasive and easily reproducible.[66] Inflammatory reactions play a crucial role in initiating endothelial dysfunction, as indicated by the correlation observed between inflammatory markers and markers of endothelial dysfunction.[54] The impairment of endothelial function leads to a cascade of events characterized by reduced endothelial nitric oxide synthase (eNOS) levels, subsequently resulting in decreased nitric oxide (NO) production;[67] the eNOS and NO reduction is associated with an increase in ET-1 production,[65,66] as demonstrated in our study. The elevation of plasma ET-1 levels correlates with reduced blood flow and increased resistance indices in the SPCAs, contributing significantly to choriocapillaris ischemia; this can subsequently lead to an increase in vascular endothelial growth factor (VEGF) production and the development of CNVM.[67,68]

Our data supports the hypothesis that the dysfunction of SPCAs and choriocapillaris endothelium, induced by a chronic subacute inflammatory process at the retinal level, is the most likely mechanism driving the onset of choriocapillaris ischemia. This hypothesis is further confirmed by the studies of Coleman et al relating to the possible efficacy of phosphodiesterase type 5 (PDE-5) and type 6 (PDE-6) inhibitors in AMD treatment.[69] These drugs have shown to improve endothelial function, increase plasma nitric oxide (NO) levels, and enhance choroidal blood flow.[70]

Regarding the mechanical theory focusing on scleral rigidity due to lipid accumulation in scleral tissue[8,71] we propose that inflammation plays a crucial role in this process. Subacute chronic inflammation would lead to an alteration of the enzymatic mechanisms that regulate the homeostasis of the extracellular matrix of the ocular tissues with consequent stiffening not only of the scleral tissue but also of the Bruch's membrane.[72–74]

Conclusion

Our data indicate that in AMD, there is a reduction in blood flow at the level of the SPCAs, accompanied by an increase of resistance index. This hemodynamic alteration is correlated with the increase in plasma ET-1 level. Alterations in scleral (SR) and corneal (CRF) biomechanics are evident only eyes with nAMD.

We think that to validate our hypotheses it would be necessary to study a greater number of patients affected by ARMD; this would allow us to better understand the trigger that leads to the appearance of ARMD and above all the possible evolution towards the neovascular form.

Disclosure

The authors declare no conflicts of interest in this work.

References

1. Nowak JZ. AMD–the retinal disease with an unprecised etiopathogenesis: in search of effective therapeutics. *Acta Pol Pharm*. 2014;71(6):900–916. PMID: 25745762.
2. Al-Zamil WM, Yassin SA. Recent developments in age-related macular degeneration: a review. *Clin Interv Aging*. 2017;12:1313–1330. PMID: 28860733; PMCID: PMC5573066. doi:10.2147/CIA.S143508
3. Deng Y, Qiao L, Du M, et al. Age-related macular degeneration: epidemiology, genetics, pathophysiology, diagnosis, and targeted therapy. *Genes&Disease*. 2021. doi:10.1016/j.gendis.2021.02.009
4. Pulido J. Scleral rigidity and macular degeneration: pathophysiologic or epiphenomenon? *Am J Ophthalmol*. 2006;141(4):731–732. PMID: 16564811. doi:10.1016/j.ajo.2005.12.021
5. Sato E, Feke GT, Menke MN, Wallace McMeel J. Retinal haemodynamics in patients with age-related macular degeneration. *Eye (Lond)*. 2006;20 (6):697–702. PMID: 15933745. doi:10.1038/sj.eye.6701951
6. Cellini M, Strobbe E, Gizzi C, Balducci N, Toschi PG, Campos EC. Endothelin-1 plasma levels and vascular endothelial dysfunction in primary open angle glaucoma. *Life Sci*. 2012;91(13–14):699–702. PMID: 22406080. doi:10.1016/j.lfs.2012.02.013
7. Finzi A, Cellini M, Strobbe E, Campos EC. ET-1 plasma levels, choroidal thickness and multifocal electroretinogram in retinitis pigmentosa. *Life Sci*. 2014;118(2):386–390. PMID: 24735956. doi:10.1016/j.lfs.2014.04.004
8. Friedman E. Update of the vascular model of AMD. *Br J Ophthalmol*. 2004;88(2):161–163. PMID: 14736760; PMCID: PMC1772021. doi:10.1136/bjo.2003.036277
9. Flaxel CJ, Adelman RA, Bailey ST, et al. Age-related macular degeneration preferred practice pattern. *Ophthalmology*. 2020;127(1):P1–P65. PMID: 31757502. doi:10.1016/j.ophtha.2019.09.024
10. Lieb WE, Cohen SM, Merton DA, Shields JA, Mitchell DG, Goldberg BB. Color Doppler imaging of the eye and orbit. Technique and normal vascular anatomy. *Arch Ophthalmol*. 1991;109(4):527–531. PMID: 2012555. doi:10.1001/archopht.1991.01080040095036
11. Guthoff RF, Berger RW, Winkler P, Helmke K, Chumbley LC. Doppler ultrasonography of the ophthalmic and central retinal vessels. *Arch Ophthalmol*. 1991;109(4):532–536. PMID: 2012556. doi:10.1001/archopht.1991.01080040100037
12. Yamamoto T, Mori K, Yasuhara T, et al. Ophthalmic artery blood flow in patients with internal carotid artery occlusion. *Br J Ophthalmol*. 2004;88 (4):505–508. PMID: 15031166; PMCID: PMC1772066. doi:10.1136/bjo.2003.025809
13. Spencer JA, Giussani DA, Moore PJ, Hanson MA. In vitro validation of Doppler indices using blood and water. *J Ultrasound Med*. 1991;10 (6):305–308. PMID: 1895370. doi:10.7863/jum.1991.10.6.305
14. Friedenwald JS. Tonometer calibration; an attempt to remove discrepancies found in the 1954 calibration scale for Schiotz tonometers. *Trans Am Acad Ophthalmol Otolaryngol*. 1957;61(1):108–122. PMID: 13409426.
15. Luce DA. Determining in vivo biomechanical properties of the cornea with an ocular response analyzer. *J Cataract Refract Surg*. 2005;31 (1):156–162. PMID: 15721708. doi:10.1016/j.jcrs.2004.10.044
16. Lau W, Pye D. A clinical description of ocular response analyzer measurements. *Invest Ophthalmol Vis Sci*. 2011;52(6):2911–2916. PMID: 21273535. doi:10.1167/iovs.10-6763
17. Nguyen BA, Reilly MA, Roberts CJ. Biomechanical contribution of the sclera to dynamic corneal response in air-puff induced deformation in human donor eyes. *Exp Eye Res*. 2020;191:107904. PMID: 31883460; PMCID: PMC7044755. doi:10.1016/j.exer.2019.107904
18. Nguyen BA, Roberts CJ, Reilly MA. Biomechanical impact of the sclera on corneal deformation response to an air-puff: a finite-element study. *Front Bioeng Biotechnol*. 2019;6:210. PMID: 30687701; PMCID: PMC6335394. doi:10.3389/fbioe.2018.00210
19. Gizzi C, Cellini M, Campos EC. In vivo assessment of changes in corneal hysteresis and lamina cribrosa position during acute intraocular pressure elevation in eyes with markedly asymmetrical glaucoma. *Clin Ophthalmol*. 2018;12:481–492. PMID: 29588569; PMCID: PMC5858548. doi:10.2147/OPTH.S151532
20. Lin CS, Kazemi A, McLaren JW, Moroi SE, Toris GB, Sit AJ. Relationship between ocular rigidity, corneal hysteresis and corneal resistance factor. *Invest Ophthalmol Vis Sci*. 2015;56:6137.
21. Glass DH, Roberts CJ, Litsky AS, Weber PA. A viscoelastic biomechanical model of the cornea describing the effect of viscosity and elasticity on hysteresis. *Invest Ophthalmol Vis Sci*. 2008;49(9):3919–3926. PMID: 18539936. doi:10.1167/iovs.07-1321
22. Sandhu R, Sivaprasad S, Shah SP, Adewoyin T, Chong NV. Pulsatile ocular blood flow in asymmetric age-related macular degeneration. *Eye (Lond)*. 2007;21(4):506–511. PMID: 16456596. doi:10.1038/sj.eye.6702242
23. Chen SJ, Cheng CY, Lee AF, Lee FL, Hsu WM, Liu JH. Pulsatile ocular blood flow of choroidal neovascularization in asymmetric age-related macular degeneration after transpupillary thermotherapy. *Eye (Lond)*. 2004;18(6):595–599. PMID: 15184925. doi:10.1038/sj.eye.6700723
24. Chen SJ, Cheng CY, Lee AF, et al. Pulsatile ocular blood flow in asymmetric exudative age related macular degeneration. *Br J Ophthalmol*. 2001;85(12):1411–1415. PMID: 11734510; PMCID: PMC1723809. doi:10.1136/bjo.85.12.1411
25. Savage HI, Hendrix JW, Peterson DC, Young H, Wilkinson CP. Differences in pulsatile ocular blood flow among three classifications of diabetic retinopathy. *Invest Ophthalmol Vis Sci*. 2004;45(12):4504–4509. PMID: 15557461. doi:10.1167/iovs.04-0077
26. Boltz A, Luksch A, Wimpissinger B, et al. Choroidal blood flow and progression of age-related macular degeneration in the fellow eye in patients with unilateral choroidal neovascularization. *Invest Ophthalmol Vis Sci*. 2010;51(8):4220–4225. PMID: 20484590. doi:10.1167/iovs.09-4968
27. Ciulla TA, Harris A, Chung HS, et al. Color Doppler imaging discloses reduced ocular blood flow velocities in nonexudative age-related macular degeneration. *Am J Ophthalmol*. 1999;128(1):75–80. PMID: 10482097. doi:10.1016/s0002-9394(99)00061-6
28. Xu W, Grunwald JE, Metelitsina TI, et al. Association of risk factors for choroidal neovascularization in age-related macular degeneration with decreased foveolar choroidal circulation. *Am J Ophthalmol*. 2010;150(1):40–47.e2. PMID: 20493466; PMCID: PMC2900527. doi:10.1016/j.ajo.2010.01.041
29. Yun C, Ahn J, Kim M, Hwang SY, Kim SW, Oh J. Ocular perfusion pressure and choroidal thickness in early age-related macular degeneration patients with reticular pseudodrusen. *Invest Ophthalmol Vis Sci*. 2016;57(15):6604–6609. PMID: 27926751. doi:10.1167/iovs.16-19989

30. Coleman DJ, Silverman RH, Rondeau MJ, Lloyd HO, Khanifar AA, Chan RV. Age-related macular degeneration: choroidal ischaemia? *Br J Ophthalmol*. 2013;97(8):1020–1023. PMID: 23740965; PMCID: PMC3717761. doi:10.1136/bjophthalmol-2013-303143
31. Leung H, Wang JJ, Rochtchina E, et al. Relationships between age, blood pressure, and retinal vessel diameters in an older population. *Invest Ophthalmol Vis Sci*. 2003;44(7):2900–2904. PMID: 12824229. doi:10.1167/iovs.02-1114
32. Friedman E, Krupsky S, Lane AM, et al. Ocular blood flow velocity in age-related macular degeneration. *Ophthalmology*. 1995;102(4):640–646. PMID: 7724181. doi:10.1016/s0161-6420(95)30974-8
33. McLeod DS, Grebe R, Bhutto I, Merges C, Baba T, Lutty GA. Relationship between RPE and choriocapillaris in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2009;50(10):4982–4991. PMID: 19357355; PMCID: PMC4829357. doi:10.1167/iovs.09-3639
34. Chirco KR, Tucker BA, Stone EM, Mullins RF. Selective accumulation of the complement membrane attack complex in aging choriocapillaris. *Exp Eye Res*. 2016:–397. PMID: 26368849; PMCID: PMC4788569. doi:10.1016/j.exer.2015.09.003
35. Chirco KR, Sohn EH, Stone EM, Tucker BA, Mullins RF. Structural and molecular changes in the aging choroid: implications for age-related macular degeneration. *Eye (Lond)*. 2017;31(1):10–25. PMID: 27716746; PMCID: PMC5233940. doi:10.1038/eye.2016.216
36. Friedman E, Ivry M, Ebert E, Glynn R, Gragoudas E, Seddon J. Increased scleral rigidity and age-related macular degeneration. *Ophthalmology*. 1989;96(1):104–108. PMID: 2919041. doi:10.1016/s0161-6420(89)32936-8
37. Pallikaris IG, Kymionis GD, Ginis HS, Kounis GA, Christodoulakis E, Tsilimbaris MK. Ocular rigidity in patients with age-related macular degeneration. *Am J Ophthalmol*. 2006;141(4):611–615. PMID: 16564793. doi:10.1016/j.ajo.2005.11.010
38. Fritsche LG, Fariss RN, Stambolian D, Abecasis GR, Curcio CA, Swaroop A. Age-related macular degeneration: genetics and biology coming together. *Annu Rev Genomics Hum Genet*. 2014;15:151–171. PMID: 24773320; PMCID: PMC4217162. doi:10.1146/annurev-genom-090413-025610
39. Gorin MB. Genetic insights into age-related macular degeneration: controversies addressing risk, causality, and therapeutics. *Mol Aspects Med*. 2012;33(4):467–486. PMID: 22561651; PMCID: PMC3392516. doi:10.1016/j.mam.2012.04.004
40. Sparrow JR, Boulton M. RPE lipofuscin and its role in retinal pathobiology. *Exp Eye Res*. 2005;80(5):595–606. PMID: 15862166. doi:10.1016/j.exer.2005.01.007
41. Zhou J, Jang YP, Kim SR, Sparrow JR. Complement activation by photooxidation products of A2E, a lipofuscin constituent of the retinal pigment epithelium. *Proc Natl Acad Sci U S A*. 2006;103(44):16182–16187. PMID: 17060630; PMCID: PMC1637557. doi:10.1073/pnas.0604255103
42. Okubo A, Rosa RH, Bunce CV, et al. The relationships of age changes in retinal pigment epithelium and Bruch's membrane. *Invest Ophthalmol Vis Sci*. 1999;40(2):443–449. PMID: 9950604.
43. Donoso LA, Kim D, Frost A, Callahan A, Hageman G. The role of inflammation in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol*. 2006;51(2):137–152. PMID: 16500214; PMCID: PMC4853913. doi:10.1016/j.survophthal.2005.12.001
44. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. *Science*. 2005;308(5720):385–389. PMID: 15761122; PMCID: PMC1512523. doi:10.1126/science.1109557
45. Telander DG. Inflammation and age-related macular degeneration (AMD). *Semin Ophthalmol*. 2011;26(3):192–197. PMID: 21609232. doi:10.3109/08820538.2011.570849
46. Rodríguez de Córdoba S, Esparza-Gordillo J, Goicoechea de Jorge E, Lopez-Trascasa M, Sánchez-Corral P. The human complement factor H: functional roles, genetic variations and disease associations. *Mol Immunol*. 2004;41(4):355–367. PMID: 15163532. doi:10.1016/j.molimm.2004.02.005
47. Johnson LV, Leitner WP, Staples MK, Anderson DH. Complement activation and inflammatory processes in Drusen formation and age-related macular degeneration. *Exp Eye Res*. 2001;73(6):887–896. PMID: 11846519. doi:10.1006/exer.2001.1094
48. Mullins RF, Russell SR, Anderson DH, Hageman GS. Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. *FASEB J*. 2000;14(7):835–846. PMID: 10783137. doi:10.1096/fasebj.14.7.835
49. Ambati J, Ambati BK, Yoo SH, Ianchulev S, Adamis AP. Age-related macular degeneration: etiology, pathogenesis, and therapeutic strategies. *Surv Ophthalmol*. 2003;48(3):257–293. PMID: 12745003. doi:10.1016/s0039-6257(03)00030-4
50. Hageman GS, Luthert PJ, Victor Chong NH, Johnson LV, Anderson DH, Mullins RF. An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. *Prog Retin Eye Res*. 2001;20(6):705–732. PMID: 11587915. doi:10.1016/s1350-9462(01)00010-6
51. Despriet DD, Klaver CC, Witteman JC, et al. Complement factor H polymorphism, complement activators, and risk of age-related macular degeneration. *JAMA*. 2006;296(3):301–309. PMID: 16849663. doi:10.1001/jama.296.3.301
52. Schaumberg DA, Hankinson SE, Guo Q, Rimm E, Hunter DJ. A prospective study of 2 major age-related macular degeneration susceptibility alleles and interactions with modifiable risk factors. *Arch Ophthalmol*. 2007;125(1):55–62. PMID: 17210852. doi:10.1001/archopht.125.1.55
53. Landowski M, Kelly U, Klingeborn M, et al. Human complement factor H Y402H polymorphism causes an age-related macular degeneration phenotype and lipoprotein dysregulation in mice. *Proc Natl Acad Sci U S A*. 2019;116(9):3703–3711. PMID: 30808757; PMCID: PMC6397537. doi:10.1073/pnas.1814014116
54. Klein R, Myers CE, Cruickshanks KJ, et al. Markers of inflammation, oxidative stress, and endothelial dysfunction and the 20-year cumulative incidence of early age-related macular degeneration: the Beaver Dam Eye Study. *JAMA Ophthalmol*. 2014;132(4):446–455. PMID: 24481424; PMCID: PMC4076038. doi:10.1001/jamaophthalmol.2013.7671
55. Fontes JD, Yamamoto JF, Larson MG, et al. Clinical correlates of change in inflammatory biomarkers: the Framingham Heart Study. *Atherosclerosis*. 2013;228(1):217–223. PMID: 23489346; PMCID: PMC3650714. doi:10.1016/j.atherosclerosis.2013.01.019
56. Salminen A, Kaarniranta K, Kauppinen A. Inflammaging: disturbed interplay between autophagy and inflammasomes. *Aging (Albany NY)*. 2012;4(3):166–175. PMID: 22411934; PMCID: PMC3348477. doi:10.18632/aging.100444
57. Wu Y, Yanase E, Feng X, Siegel MM, Sparrow JR. Structural characterization of bisretinoid A2E photocleavage products and implications for age-related macular degeneration. *Proc Natl Acad Sci U S A*. 2010;107(16):7275–7280. PMID: 20368460; PMCID: PMC2867734. doi:10.1073/pnas.0913112107
58. Radu RA, Hu J, Yuan Q, et al. Complement system dysregulation and inflammation in the retinal pigment epithelium of a mouse model for Stargardt macular degeneration. *J Biol Chem*. 2011;286(21):18593–18601. PMID: 21464132; PMCID: PMC3099675. doi:10.1074/jbc.M110.191866

59. Schroder K, Tschopp J. The inflammasomes. *Cell*. 2010;140(6):821–832. PMID: 20303873. doi:10.1016/j.cell.2010.01.040
60. Jang YP, Matsuda H, Itagaki Y, Nakanishi K, Sparrow JR. Characterization of peroxy-A2E and furan-A2E photooxidation products and detection in human and mouse retinal pigment epithelial cell lipofuscin. *J Biol Chem*. 2005;280(48):39732–39739. PMID: 16186115. doi:10.1074/jbc.M504933200
61. Eldred GE. Lipofuscin fluorophore inhibits lysosomal protein degradation and may cause early stages of macular degeneration. *Gerontology*. 1995;41(2):15–28. PMID: 8821318. doi:10.1159/000213722
62. Narayan S, Prasanna G, Krishnamoorthy RR, Zhang X, Yorio T. Endothelin-1 synthesis and secretion in human retinal pigment epithelial cells (ARPE-19): differential regulation by cholinergics and TNF-alpha. *Invest Ophthalmol Vis Sci*. 2003;44(11):4885–4894. PMID: 14578413. doi:10.1167/iovs.03-0387
63. Skeie JM, Fingert JH, Russell SR, Stone EM, Mullins RF. Complement component C5a activates ICAM-1 expression on human choroidal endothelial cells. *Invest Ophthalmol Vis Sci*. 2010;51(10):5336–5342. PMID: 20484595; PMCID: PMC3066598. doi:10.1167/iovs.10-5322
64. Rodrigo F, Ruiz-Moreno JM, García JB, Torregrosa ME, Segura JV, Piñero DP. Color Doppler imaging of the retrobulbar circulation and plasmatic biomarkers of vascular risk in age-related macular degeneration: a pilot study. *Indian J Ophthalmol*. 2018;66(1):89–93. PMID: 29283130; PMCID: PMC5778590. doi:10.4103/ijo.IJO_488_17
65. Wang R, Wang Y, Mu N, et al. Activation of NLRP3 inflammasomes contributes to hyperhomocysteinemia-aggravated inflammation and atherosclerosis in apoE-deficient mice. *Lab Invest*. 2017;97(8):922–934. PMID: 28394319; PMCID: PMC5537437. doi:10.1038/labinvest.2017.30
66. Baltu F, Sarici AM, Yildirim O, Mergen B, Bolat E. Investigation of vascular endothelial dysfunction in the patients with age-related macular degeneration. *Cutan Ocul Toxicol*. 2019;38(1):29–35. PMID: 30037291. doi:10.1080/15569527.2018.1504056
67. Totan Y, Koca C, Erdurmuş M, Keskin U, Yiğitoğlu R. Endothelin-1 and nitric oxide levels in exudative age-related macular degeneration. *J Ophthalmic Vis Res*. 2015;10(2):151–154. PMID: 26425317; PMCID: PMC4568612. doi:10.4103/2008-322X.163765
68. Sakai S, Yorikane R, Miyauchi T, et al. Altered production of endothelin-1 in the hypertrophied rat heart. *J Cardiovasc Pharmacol*. 1995;26(3):S452–5. PMID: 8587443. doi:10.1097/00005344-199526003-00133
69. Coleman DJ, Lee W, Chang S, et al. Treatment of macular degeneration with sildenafil: results of a two-year trial. *Ophthalmologica*. 2018;240(1):45–54. PMID: 29694963; PMCID: PMC7597673. doi:10.1159/000486105
70. Deyoung L, Chung E, Kovac JR, Romano W, Brock GB. Daily use of sildenafil improves endothelial function in men with type 2 diabetes. *J Androl*. 2012;33(2):176–180. PMID: 21680809. doi:10.2164/jandrol.111.013367
71. Broekhuyse RM. The lipid composition of aging sclera and cornea. *Ophthalmologica*. 1975;171(1):82–85. PMID: 1124206. doi:10.1159/000307448
72. Liu H, Xiang N, Zhang H. Influence of high level TGF-beta1 on scleral thickness. *J Huazhong Univ Sci Technolog Med Sci*. 2007;27(5):601–604. PMID: 18060646. doi:10.1007/s11596-007-0532-0
73. Dewing JM, Carare RO, Lotery AJ, Ratnayaka JA. The diverse roles of TIMP-3: insights into degenerative diseases of the senescent retina and brain. *Cells*. 2019;9(1):39. PMID: 31877820; PMCID: PMC7017234. doi:10.3390/cells9010039
74. Strickland RG, Garner MA, Gross AK, Girkin CA. Remodeling of the lamina cribrosa: mechanisms and potential therapeutic approaches for glaucoma. *Int J Mol Sci*. 2022;23(15):8068. PMID: 35897642; PMCID: PMC9329908. doi:10.3390/ijms23158068