GRIGORE T. POPA UNIVERSITY OF MEDICINE AND PHARMACY IASI

New diagnostic and therapeutic approaches to ocular diseases

HABILITATION THESIS

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CONTENTS

ABBREVIATIONS LIST

CNV	Choroidal neovascularization
SIO	Silicone oil
CRF	Case Report Form
DME	Diabetic Macular Edema
OCT	Optical Coherence Tomography
OCT-A	Optical Coherence Tomography Angiography
MNV	Macular Neovascularization
SLT	Selective Laser Trabeculoplasty
Anti-VEGF	Anti-Vascular Endothelial Growth Factors
AMD	Age Related Macular Degeneration
RPE	Retinal Pigment Epithelium
RP	Retinitis Pigmentosa
LCA	Leber Congenital Amaurosis
IK	Infectious Keratitis
OSD	Ocular Surface Diseases
CXL	Collagen Cross-Linking
CF	Conjunctival flap
AMT	Amniotic membrane transplantation
PACK-CXL	Conventional Corneal Collagen CXL
PACKA-CXL	Accelerated Corneal Collagen CXL
OCP	Ocular cicatricial pemphigoid
IVCM	In vivo confocal microscopy
CFs	Conjunctival flap surgery
AM	Amniotic membrane
PSEDs	Posterior Segment Eye Diseases
AOO	Adaptive optics ophthalmoscopy
AO	Adaptive optics
AI	Artificial intelligence
CSC	Central serous chorioretinopathy
PPE	Pachychoroid pigment epitheliopathy;
PNV	Pachychoroid neovasculopathy
DM	Diabetes Mellitus
BCVA	Best-corrected visual acuity
ROP	Retinopathy of prematurity
MLC	Machine learning classes
APMPPE	Acute posterior multifocal placoid pigment epitheliopathy
DR	Diabetic retinopathy
UM	Uveal melanoma
GT	Gene therapy
NVG	Neovascular glaucoma
IOP	Intraocular pressure
TSCPC	Transscleral cyclophotocoagulation
CW-TSCPC	Continuous-wave transscleral cyclophotocoagulation
MP-TSCPC	Micropulse transscleral cyclophotocoagulation
AJCC	American Joint Committee on Cancer Classification
UBM	Ultrasound biomicroscopy

COMS	Collaborative Ocular Melanoma Study
FA	Fluorescein angiography
ICG	Indocyanine green angiography
FNAB	Fine-needle aspiration biopsy
GEP	Gene expression profiling
TTT	Transpupillary thermotherapy
PDT	Photodynamic therapy
AAV	Adeno-associated viruses
MAC	Membrane attack complex
PPV	Pars plana vitrectomy
MIVS	Microincision vitrectomy surgery
IOL	Intraocular lens
SRH	Subretinal hemorrhage
rTPA	Recombinant tissue plasminogen activator
PFCLs	Perfluorocarbon liquids
SRH	Subretinal hemorrhage
PDS	Port delivery system
RGC	Retinal ganglion cells
TM	Trabecular meshwork
OS	Oxidative stress
DNA	Deoxyribonucleic acid

THESIS SUMMARY

Combining the medical and didactic careers is a challenge that admits no compromises. Reaching professional skills is mandatory and this represents the foundation for the didactic career, gradually improvable through self-improvement within the academic strictness. During this time, active involvement in professional and educational activities is imperative to gain individuality and visibility.

This habilitation thesis reviews my professional, academic and scientific activity during the postdoctoral period (2006-2021) at the "Grigore T. Popa" University of Medicine and Pharmacy in Iași. As suggested by the National Council for Attestation of Titles, Diplomas, and Certificates (CNATDCU), the present thesis is structured in four sections, as follows:

Section I offers a summary of my professional, academic, and scientific achievements.

Section II presents the most important directions of my postdoctoral research activity, focused on various diagnostic methods, medical therapies, and surgical techniques in various ocular diseases. This section is structured into 2 chapters that highlight the most important areas of interest:

Chapter 1, generically entitled “Modern diagnosis, therapies and updates in various ocular diseases and ocular involvements”, is further subclassified into 4 sections:

The first section is entitled “New diagnostic and therapeutic techniques in ocular surface pathology” and is presenting in detail researches focusing on various diseases such as ocular cicatricial pemphigoid, diagnostic methods for the etiological assessment of infectious corneal pathology, the indications and efficacy of the conjunctival flap surgery and the amniotic membrane in the management of the ocular surface disease. Clinical research concerning the efficacy and safety of a novel collagen cross-linking technique for the treatment of infectious keratitis is also presented.

The second section of the first chapter is entitled “New concepts and diagnostic methods in posterior segment eye diseases” and includes researches on the current diagnosis and management strategies in the pachychoroid spectrum of diseases and also on the present and future of the artificial intelligence and deep learning in ophthalmology. This section also includes some of the research on new imaging tools for various retinal pathologies. In particular, the significance of spectral-domain optical coherence tomography in the diagnosis and treatment decision of some rare cases is highlighted as also the importance of retinal microcirculation investigation in diabetic patients using an adaptive optics retinal camera.

The third section of the first chapter is entitled “New therapeutic options in posterior segment eye diseases” and includes researches on uveal melanoma diagnosis and current treatment options, current trends in gene therapy for retinal diseases, and a clinical study comparing the efficacy and safety of micropulse vs. continuous-wave transscleral cyclophotocoagulation in neovascular glaucoma of various etiologies.

The fourth section of the first chapter is entitled “Modern vitreoretinal surgery: controversies and a critical evaluation of different techniques and adjuncts”. In this subchapter, the results of various researches on modern therapies are presented, such as the efficacy and safety of subretinal alteplase injections in massive subretinal hemorrhage due to neovascular age-related macular degeneration and the outcomes of simultaneous vs. sequential pars plana vitrectomy and cataract surgery. There are also included studies concerning the anatomical and functional results following vitreoretinal surgery for various complications of the diabetic retinopathy, anatomical results and complications

after silicone oil removal, and the intraocular pressure changes during and after silicone oil endotamponade.

The second chapter entitled "Anti-VEGF agents: the revolutionary treatment paradigm shift in neovascular retinal diseases" is dedicated to the most innovative therapeutic option of the last decade that has significantly improved the anatomical and functional outcomes in many retinal diseases. The ethical aspects of the "off-label" bevacizumab (Avastin®) usage in Romania for almost 10 years are discussed. The chapter includes researches on the efficacy and safety of this treatment in choroidal neovascularization secondary to less frequent diseases, such as high myopia and angioid streaks. There are also presented researches on real-life management of refractory choroidal neovascularization using various intravitreal agents and also the influence of intravitreal drug administration on the intraocular pressure and the incidence of choroidal neovascularization in the fellow eye.

Section III includes the most important personal tasks for the professional and academic activity in the future. Among them, of major importance are the constant improvement in expertise through new diagnostic and therapies, and the active participation in medical meetings. The multidisciplinary approach and the high-quality, attractive courses for residents and young specialists are also very important in my opinion. I encourage young specialists to be actively involved in national and international medical meetings and to maintain a positive spirit in the Discipline, based on respect and equal opportunity.

The research activity will continue to represent a significant proportion in the future. Some of the future research projects, like the importance of targeting the oxidative stress in glaucoma, the implications of genetic polymorphism associated with thromboembolic risk in various ocular diseases, the efficacy of micropulse transscleral cyclophotocoagulation for glaucoma after penetrating keratoplasty and differences and similarities between cutaneous and uveal melanoma are already in an advanced stage of refinement and will be soon published.

Section IV comprises the list of references included in the thesis.

REZUMATUL TEZEI

Asocierea carierei didactice cu cea medicală reprezintă o provocare care nu acceptă compromisuri. Atingerea performanței profesionale este absolut necesară și reprezintă temelia pe care se clădește gradual cariera didactică, în rigorile mediului academic, prin dezvoltare personală continuă. În tot acest timp, implicarea activă în manifestări profesionale și educaționale este esențială, alături de activitatea de cercetare, pentru a conferi individualitate și vizibilitate.

Această teză de abilitare trece în revistă activitatea mea profesională, academică și științifică din perioada postdoctorală (2006-2021), în cadrul Universității de Medicină și Farmacie „Grigore T. Popa" din Iași. Conform recomandărilor Consiliul Național pentru Atestarea Titlurilor, Diplomelor și Certificatelor (CNATDCU), prezența teză este structurată în patru secțiuni, după cum urmează:

Prima secțiune sumarizează realizările profesionale, academice și științifice.

Secțiunea a II-a prezintă cele mai importante direcții de cercetare postdoctorală, centrate pe diverse metode diagnostice, terapii medicamentoase și chirurgicale în diverse patologii oculare. Această secțiune este structurată în 2 capitole ce ilustrează principalele zone de interes :

Capitolul 1, intitulat generic "Diagnosticul și terapiile moderne în diverse boli oculare sau atingeri oculare" este la rândul lui subclasificat în 4 secțiuni :

O primă secțiune intitulată "Noi metode diagnostice și terapeutice ale patologiei suprafeței oculare" detaliază cercetări referitoare la pemfigoidul ocular cicatricial, eficacitatea diverselor metode diagnostice în evaluarea etiologică a patologiei corneene infecțioase, indicațiile și eficacitatea voletului conjunctival și a membranei amniotice în managementul diverselor patologii oculare. Este inclus, de asemenea, un studiu clinic comparativ referitor la eficacitatea și siguranța unei variante moderne de cross-linking în tratamentul keratitelor infecțioase.

Cea de a doua secțiune a primului capitol este intitulată "Noi concepte și metode diagnostice în patologia segmentului posterior ocular" și include cercetări referitoare la conceptul modern al spectrului de boli ale pahicoroidei precum și inteligența artificială și învățarea profundă în diverse patologii oculare. În acest subcapitol sunt incluse și o parte din cercetările clinice bazate pe aportul unor metode imagistice moderne în diverse patologii retiniene. Este detaliat rolul tomografiei în coerentă optică spectrală în diagnosticul și tratamentul unor cazuri clinice rare și importanța evaluării microcirculatiei retiniene prin metoda opticii adaptative la pacienții diabetici fără retinopatie.

Cea de a treia secțiune a primului capitol este intitulată "Noi opțiuni terapeutice în patologia segmentului posterior ocular" și include cercetări referitoare la diagnosticul și opțiunile terapeutice moderne ale melanomul malign coroidian, terapiile genice în bolile retinei dar și studiul clinic referitoare la evaluarea comparativa a eficacității și siguranței terapiei laser (micropulsat sau continuu) în glaucomul neovascular de diverse etiologii.

Cea de a patra secțiune a primului capitol este intitulată "Chirurgia vitreoretiniană modernă: controverse și evaluarea critică a diverselor tehnici și adjuvanți". În acest subcapitol sunt prezentate rezultatele unor cercetări care vizează abordări terapeutice moderne, precum eficacitatea și siguranța utilizării de altepază subretinian în hemoragiile subretiniene masive din degenerescența maculară legată de vârstă neovasculară dar și compararea chirurgiei simultane (la nivelul segmentului anterior și posterior) cu cea seriată în diverse patologii retiniene, Sunt, de asemenea, incluse rezultatele anatomice și funcționale obținute după chirurgia diverselor complicații ale retinopatiei diabetice, rezultatele anatomice și complicațiile după ablația uleiului siliconic și un studiu referitor la fluctuațiile presiunii intraoculare în timpul și după endotamponada siliconica.

Capitolul al 2-lea este dedicat celei mai recente și importante achiziții terapeutice in retinologie, care a ameliorat semnificativ prognosticul anatomic și funcțional în multe patologii retiniene și este intitulat "Schimbarea revoluționară de către agenții Anti-VEGF a paradigmei terapeutice în bolile neovasculare ale retinei". Este abordat aspectul etic al utilizării bevacizumabului (Avastin®) în România în sistem « off-label » pentru mai bine de un deceniu și sunt prezentate rezultatele unor cercetări clinice care au vizat evaluarea eficacității și siguranței acestuia in tratamentul neovascularizatiei maculare asociată unor patologii retiniene mai puțin frecvente precum miopia degenerativă și striurile angioide. Sunt de asemenea, prezentate rezultatele unor cercetări asupra abordării, în viață reală, a neovascularizatiei coroidiene refractare utilizand si alti agenti in injectare intravitreana dar și impactul acestui tratament asupra presiunii intraoculare sau incidenței neovascularizatiei maculare la ochiul congener.

În Secțiunea a III-a sunt menționate cele mai importante obiective de viitor in activitatea profesionala și academica. Dintre acestea, ameliorarea continuă a expertizei prin însușirea și implementarea de noi metode diagnostice și terapeutice, alături de menținerea unei prezențe active la manifestările de specialitate reprezintă obiective prioritare. Abordarea multidiciplinară și îmbunătățirea calității pregătirii medicior rezidenți și tineri specialiști prin susținerea de cursuri de educație medicală atractive, la un nivel științific ridicat, reprezintă, de asemenea, repere importante ale activității mele didactice ulterioare. Nu în ultimul rând, îmi propun încurajarea prezenței active a tinerilor la manifestările științifice naționale și internaționale alături de menținerea unei atitudini pozitive în cadrul colectivului disciplinei, bazat pe respect și egalitatea de șansă.

În ceea ce privește activitatea de cercetare ea va continua să reprezinte o pondere importantă. Cîteva dintre proiectele viitoare de cercetare, precum cele legate de importanța stresului oxidativ în glaucom, polimorfismul genic asociat riscului tromboembolic in diferite boli oculare, eficacitatea ciclofotocoagularii transsclerale in glaucomul postkeratoplastie și diferențele dintre melanomul cutanat și cel uveal se găsesc deja într-un stadiu avansat de elaborare și urmează să fie publicate în curînd.

Secțiunea a IV-a include lista referințelor bibliografice menționate în teza de abilitare.

SECTION I
CHAPTER 1. PROFESSIONAL, ACADEMIC, AND SCIENTIFIC ACHIEVEMENTS

Life is a constant source of opportunities. For those few who have succeeded in medical school a significant number of chances are suddenly revealed. Among these, embracing an academic career in addition to a medical career is one of the most challenging. The long road, often full of unexpected obstacles, makes one easily balance from agony to ecstasy. There is a tremendous need for self-education and constant improvement in both the medical profession and teaching skills. While not always rewarding, the academic career requests strength and patience sometimes against all odds. The time often excessive spent with patients during training years, the increasing experience due to numerous challenging medical and surgical procedures, and the bonding with the audience during classes and lectures will represent the key ingredients in transforming you into a valuable asset in teaching. Also, together with experience and knowledge comes the encouragement for research and the inspiration for scientific publications.

1.1.Professional achievements

In 1992, immediately after graduation, I started my training in ophthalmology, as a resident, in the Ophthalmology Clinic of "Sf. Spiridon" Hospital Iasi, Romania, by order of the Romanian Minister of Health No. 1228/21.12.1992.

In 1995, after overpassing the final residency examination, I was entitled a Specialist in Ophthalmology (Ministry of Health Confirmation No. 2393/18.12.1995) and worked between 1995 and 2000 in the Ophthalmology Clinic, Hospital "Sf. Spiridon" Iasi.

In 2000, after a new evaluation, I was entitled Senior Ophthalmologist (Ministry of Health Confirmation No 727/07.09.2000) and continued to work in the same Ophthalmology Clinic, Hospital "Sf. Spiridon" Iasi, with clinical integration, until 2011.

From 2011 until today I perform outpatient ophthalmological consultations and different treatment procedures (intravitreal injections, laser therapies, and vitreoretinal surgery) in the private setting of "Retina Center" Eye Clinic, Iasi.

Immediately after completing my residency, I have accomplished multiple fellowships to obtain expertise in medical, laser, and surgical retinal pathology.

In 1995, in Lyon, France, I have assisted Prof. Dr. Jean-Daniel Grange at the "Clinique Ophtalmologique Universitaire à l'Hôpital de la Croix Rousse" in the field of conservative treatments for various ocular tumours (brachiterapy and protontherapy) and Dr. Jean Tavernier in the outpatient Clinic of "Centre Hospitalier St Joseph & St Luc".

In 1996, with the generous support of the Humana Foundation, I had the unique opportunity to follow experts in retinology as Dr.John Gamel, *Professor Emeritus* at the University of Louisville, Kentucky (in the field of retinal imaging and pathology), and Dr.Keneth Jaeger (in retinal laser therapies and retinal surgery) at Kentucky Lions Eye Center and Veterans Hospital. I have also spent significant time closely following Prof.Dr.Charles C.Barr, Professor of ophthalmology and visual sciences and director of the Retina Service at the University of Louisville, in both clinical field and also in teaching at Kentucky Lions Eye Center and Jewish Hospital,

At the end of 1996, I returned in Lyon, France at "Clinique Ophtalmologique Universitaire à l'Hôpital de la Croix Rousse", to complete one year of "AFSA" (Attestation de Formation Spécialisée Approfondie) in the field of retinal pathology in the

Ophthalmology Department of Claude Bernard University. During that year I worked mainly in the retinal pathology and intraocular tumors under the direct supervision of Prof. Dr. Mireille Bonnet and Prof. Dr. Jean-Daniel Grange.

Upon my return home in 1997, with the generous financial support from University "Grigore T.Popa" Iasi, Humana Foundation from the USA, and the equipment donated from Lyon, France, I have started the systematic implementation of laser therapy and surgical procedures (scleral buckling and pars plana vitrectomies) for various retinal disorders under the close monitoring of my former teachers from the USA that came to Romania on this occasion. Thus, Iasi became the second national medical center, after Bucharest, able to perform advanced evaluation and modern medical, laser, and surgical procedures in patients with various retinal pathologies.

In 1999 I followed additional fellowships in the Ophthalmology Department of Ghent University, Belgium, under the supervision of Prof.Dr. Jean-Jaques De Laey (in the field of retinal angiographic evaluation), and Prof. S.Verbraken (in the field of retinal surgery).

Also, in 1999 and later in 2000 I completed fellowships in the Ophthalmology Departments of Freie Univerity Berlin, under the supervision of Prof. Dr. Michael Foerster, and in Würzburg, with Prof. Dr. med Dr. hc. Franz Grehn and Prof. Dr. Wolfgang Schrader in the fields of ocular trauma and retinal surgery.

Since 2000, in the Ophthalmological Clinic at "Sf. Spiridon" Hospital I have performed thousands of surgical procedures for retinal detachments, vitreous hemorrhages of different etiology, macular holes or epiretinal membranes, ocular trauma with or without intraocular foreign bodies, dislocated intraocular lenses, or severe intraocular infections. Also, a tremendous number of retinal laser procedures were performed, especially in proliferative diabetic retinopathy and macular edema, retinal breaks, and retinal vascular occlusions.

In the field of retinal imaging, retinal photography and fluorescein angiography have been implemented and systematically performed since the first clinical trials were initiated in the Clinic, under my guidance, in the early 2000s.

Since 2000 I have followed further fellowships in Frankfurt, Milano, Prague, Barcelona, Vienna, Lisbon, Cannes, and Warsaw.

I initiated, in the early 2000s, with the support of the Diabetes Clinic Director, a long-term screening program with the Diabetology Department to facilitate a better early diagnosis, treatment, and follow-up of patients with diabetic retinopathy and other ocular complications related to diabetes mellitus.

As previously mentioned, my professional activity continues since 2011, when I resigned from the Ophthalmology Clinic of "Sf.Spiridon" Hospital due to irreconcilable differences of opinion, in the private setting of "Retina Center" Eye Clinic Iasi. Here I continue to perform outpatient consultations, mainly in the field of retinal pathologies, and also modern ambulatory micro-incisional vitreoretinal surgery, various laser procedures, and novel intravitreal therapies. I have never stopped improving my knowledge and professional skills while actively participating in numerous online and onsite training programs.

Since my early professional beginnings, I joined national and international scientific societies. I am currently holding the position of board member in the Romanian Society of Ophthalmology (for the second consecutive mandate) and also in the Romanian RETINA Society, in which I am a co-founding member. I am also a member of EURETINA (European Society of Retina Specialists), SFRETINE (Société Française de Rétine), European Laser Association, Romanian Society of Cataract and Refractive Surgery, and The Society of Physicians and Naturalists of Iaşi.

In 2014, as a result of my professional evaluation by the European Board of Ophthalmology and with the generous assistance of the Directing Council of the Romanian Society of Ophthalmology I was entitled „FEBO *ad eundem*" (Fellow of the European Board of Ophthalmology) for my consistent contribution in implementing and development of Romanian Retinology.

1.2. Academic career

My teaching career in the Ophthalmology Department at the University of Medicine and Pharmacy "Grigore T.Popa" Iasi started in September 1991, soon after graduation, after a competitive examination, as a Junior Teaching Assistant for the Faculty of Medicine. In October 1994, after a new competitive examination, I became University Assistant for the same Faculty. During that time, the burden of the numerous classes to be held by a limited number of teaching assistants and in inadequate teaching conditions was very high. The increasing number of students and the implementation of a new study program, in the English language, were ultimately managed using a schematic, simplified educational plan.

The professional experiences from abroad often implied teaching and this encouraged me to implement, on my return, a more active approach, based on suggestive iconography and interaction. I still have in my library the sheets with slides I have bought from France, Belgium, Germany, and the United States and used during lectures and classes with students and residents to illustrate different ocular pathologies. Hopefully, the digital systems quickly evolved in 2000 so I was able to implement and systematically illustrate the lectures with cases from the outpatient clinic and also with different surgical techniques.

In February 2008, after long waiting, I was finally accepted to advance to the position of Lecturer in Ophthalmology and officially granted for lectures with the students. As expected, my decision to present the lectures for students in an earlier manner than used to, in the first consecutive 7 weeks of the semester was in the beginning regarded with plenty of skepticism. But, as a result, it was very well appreciated by the students and increased the general interest in ophthalmology. Noticing that the students are filling back the lecture halls, although this was not mandatory for overpassing the final examination was extremely rewarding.

One of the most challenging moments in my teaching career was in 2011, when, soon after the academic year started, I was requested by the University Board to take over the lectures on the newly established study program in French, first for the Faculty of Dental Medicine and soon after that for the Faculty of Medicine. In less than one semester I was able to successfully adapt and finalize both the lectures and also the practical classes. The summary was adjusted to also include specific topics required by the French Ophthalmology University College and is updated regularly from that moment on.

I have actively contributed, during the years, with personal chapters, in several editions of student books for both practical and oral evaluation. I have also coordinated numerous bachelor's degree theses for medical students, including for students from the study program in French.

Since January 2021 I advanced as Associate Professor at the Ophthalmology Department at the University of Medicine and Pharmacy "Grigore T.Popa" Iasi.

During my entire teaching career, I was actively involved in training several generations of young residents in ophthalmology mainly in retinal pathology, modern retinal imaging techniques, specific treatments, and retinal surgery. Together with my fellow retina specialists from Romania and abroad and with the support of the Romanian RETINA Society, Romanian Ophthalmological Society, Romanian College of Physicians,

and the Dermatological Association from Moldavia, numerous successful teaching courses for residents and young ophthalmologists were organized. During the years, I have also coordinated many original papers presented by residents at national or international conferences.

I am constantly involved in different organizing committees of national conferences and requested to join different commissions responsible for bachelor programs, master programs, specialist/senior physician titles, presentations of scientific reports within the doctoral thesis, and didactic contests.

Due to my expertise in retinology, I am frequently asked, to participate, as a national key opinion leader, in local, national, and international industry symposiums, conferences and congresses, different webinars and teachings programs, and also in multidisciplinary meetings.

1.3 Research activities

The year 1996 represented a landmark in my research activity as I was able to qualify as a co-investigator in the international clinical study entitled "A Double-blind placebo-controlled study of calcium dobesilate (Doxium®) in the treatment of mild to moderate diabetic retinopathy". The study lasted from 1996 to 2003 and successfully ended. In the next years, due to favorable recommendations, I was requested to join, as an investigator, in many more international prospective randomized clinical studies such as:

•"A randomized, controlled study on the efficacy and safety of Sandostatin LAR in the therapy of patients with moderately to severe or severe non-proliferative diabetic retinopathy or low risk proliferative diabetic retinopathy" – SANDOSTATIN LAR study, 2000-2005; Certificate in "Seven-field stereo retinal photography" by the Department of Ophthalmology and Visual Sciences, University of Wisconsin-Madison

•"A randomized, multicenter, double-blind placebo-controlled comparative phase III study in outpatients with mild to moderate non-proliferative diabetic retinopathy to assess the efficacy of treatment with a once-daily administration of one 600mg lipoic acid bollet for 24 months" – RETIPON study, 2000-2005;

•"A phase III randomized multicenter, multinational, double-masked, placebo-controlled study of PHOTREX (Rostaporfin) Photodynamic therapy in the treatment of classic and occult subfoveal Choroidal neovascularization associated with age-related macular degeneration" - PHOTREX study, 2005;

•"Effects of Candesartan Cilexetil (Candesartan) on Diabetic Retinopathy in Type 1 Diabetic Patients Without Retinopathy" – DIRECT study, 2001-2008; Certificate DIR-292, in "Seven-field stereo retinal photography"

•"Long term double-blind comparison of gliclazide modified release and an oral anti-diabetic given in combination with metformin in type two diabetic patients: a two year international, multicentre, randomized, double-blind, parallel-group study followed by a two-year double-blind extension" - ENDORSE Study, 2006-2008;

•"Retrospective study to evaluate the patient demographic, disease characteristic, treatment pattern, and healthCare resource utilization in patients with active non-infectious, intermediate, pOsterior or Panuveitis in specialized ophthalmological clinical cEnters – eyeCOPE", 2017-2018.

Also, between 2006 and 2013 I participated in 3 national research projects (Project CNCSIS, contract no. 31GR/2007, Grant CEEX, contract 192/2006, and the Project POSDRU 31081/2010).

The active involvement in clinical studies and research projects seemed to be mandatory for the development of teamwork abilities, understanding of the protocol discipline, and the implementation of rigorousness to all data obtained.

In 2000 I decided to start working on the Doctoral Thesis under the guidance of the scientific coordinator, Prof. Dr. Dorin Chiselita. As most of my daily practice consisted at that time of surgery of diabetic retinopathy complications I have decided to contribute to elucidating some of the controversies regarding the efficacy and safety of different surgical techniques. Thus, a series of dedicated retrospective, prospective and interventional studies were initiated to validate the efficacy (through the anatomical and functional results) and safety (through the incidence and severity of intra and postoperative complications) of different surgical techniques in patients vitrectomized for diabetic retinopathy complications. The study group included 321 eyes from 296 patients operated exclusively by the author, under local anesthesia, between 1st of January 2000 and 1st of January 2005 in the Ophthalmology Clinic of "St.Spiridon" Hospital Iasi. State of the art surgical equipment at that time was used (20G Alcon Accurus vitrectomy unit with Ophthalas 532 Eyelite laser photocoagulator and Moeller-Weddel HiR9 operating microscope). Wilcoxon, Anova, correlation, and regression testing were performed for statistical analysis which allowed the global data evaluation and also the sub-group detailed analysis according to surgery indication and technique.

The results were presented in 2006 and the Doctoral Thesis entitled "Contributions at the comparative study of different modern vitreoretinal surgery techniques in the diabetic eye" was highly appreciated and validated under Minister Order no 3824/03.05.2006. The main conclusions of the Thesis can be resumed as follow:

- ✓ The benefit of local subtenonian anesthesia resulted from the intraoperative comfort and quick postoperative recovery during 20G vitrectomy;
- ✓ Intra and postoperative adjuncts and modern instrumentation were mandatory to successfully manage more complex cases including recurrences;
- ✓ Regarding different surgical techniques, in my experience, the efficacy of viscodissection and "en bloc" excisions was confirmed in the case of small to moderate only preretinal membranes without highly vascularized retinal proliferations;
- ✓ Air endotamponade prevented early vitreous hemorrhage recurrence after vitrectomy but enhanced cataract formation so it should be electively used in pseudophakic or aphakic eyes only;
- ✓ The prospective study on refractory DME revealed an anatomical improvement after vitrectomy but limited vision gain if the patient had more than 3 previous macular laser photocoagulation sessions;
- ✓ No statistically significant influence on the long-term values of intraocular pressure was noticed after vitrectomy.

The research activity can also be highlighted by the published articles. Up to this moment, I have contributed, as main author or co-author to 187 scientific reports. Among these, 76 have been published *in extenso* and 111 in abstract books (74 in national abstract books and 37 in international abstract books).

I have also elaborated as main author or co-author in 30 book chapters, including textbooks for students and residents.

To date, a number of 33 articles with ISI Thomson indexing (reaching so far a Hirsch index of 8), have been published. Also, 39 more articles to date are published in journals indexed in international databases. The experience gained in research and publishing allowed me to be selected as Assistant Editor and member of the National Editorial Board of the "Romanian Journal of Ophthalmology" (Online ISSN: 2501-2533, ISSN-L 2457-4325, Print ISSN 2457 – 4325, ISSN-L 2457 – 4325), reviewer at "Special Issue of Experimental and Therapeutic Medicine" (Print ISSN: 1792-0981, Online ISSN:

1792-1015, Current Impact Factor 2.447) and reviewer at "Acta Endocrinologica" (Print ISSN: 1841-0987, Online ISSN: 1843-066X, Current Impact Factor 0.73).

During the years I have provided 242 presentations and lectures at different national and international conferences, as an invited speaker in different symposia, and in various postuniversitary and continuing medical education courses.

SECTION II
SCIENTIFIC ACHIEVEMENTS

Chapter 1. Modern diagnosis, therapies, and updates in various ocular diseases and ocular involvements.

1.1. State of the art and scientific context.

As in most medical branches, recent decades, and in particular, the last few ones had a major impact on ophthalmology practice from the point of view of both diagnostic and therapies. The revolution in ophthalmological technology, instrumentation, and resources happened at all levels and continues every day. At a very quick glance, some of these major achievements in the diagnosis, medical, and surgical treatment of major eye diseases will be mentioned to frame the current state of the art and the scientific context.

Cataract surgery is currently assisted by laser, which is much quicker and safer and the visual restoration due to newer intraocular lenses is much better for near, intermediate, and distance vision.

The classic technique of radial keratotomy has been successively replaced by several generations of laser techniques that are nowadays the standard in corneal refractive surgery. Corneal transplantation, once represented by the full-thickness penetrating keratoplasty only, has undergone improvements not only in instrumentation but in newer techniques such as deep anterior lamellar keratoplasty, lamellar keratoplasty, and Descemet's stripping automated endothelial keratoplasty. Moreover, in keratoconus, corneal collagen cross-linking with combined riboflavin ophthalmic solutions and ultraviolet light irradiation is available.

In glaucoma, the topical medical armamentarium has continuously evolved and enlarged through newer prostaglandins, alpha2-adrenergic agents, and carbon anhydrase inhibitors. Preservative-free formulas are associated with fewer adverse events and better compliance. The classical argon laser trabeculoplasty performed in primary open-angle glaucoma is nowadays almost completely replaced by the more effective SLT.

In the field of posterior segment procedures, the continuous improvement of vitrectomy techniques, instrumentation, and surgical adjuncts, made the surgical procedures more efficient, safer, less invasive, and allowed for treating most of the "untreatable" cases. And this is not the only major achievement in posterior segment pathologies. The intravitreal administration of various anti-VEGF agents or steroidal implants is the new standard of care and replaced laser therapy in neovascular AMD and most retinal vascular diseases. Still, laser photocoagulation keeps evolving with newer, more efficient, and less invasive techniques such as the pattern, navigated, and the subthreshold micropulse laser.

Besides different anti-VEGF agents, ocular gene therapy is nowadays available for RPE65-related Leber congenital amaurosis (LCA) or retinitis pigmentosa (RP), which induces severe vision loss since early childhood.

Optical coherence tomography (OCT) and Optical coherence tomography angiography (OCT-A) are the most important achievements in retinal imaging for various diseases including glaucoma. These non-invasive techniques are nowadays mandatory for diagnosis, treatment decisions, and follow-up, due to the outstanding images of the macular retinal architecture including choriocapillaris, the superficial and deep retinal capillary plexus.

Almost none of these achievements would have been possible without consistent research. Today, in the era of evidence-based medicine, randomized clinical trials are the

gold standard for evaluating therapies on both efficacy and safety. Due to the enormous costs, multiple phases, infrastructure, and unique designs, randomized clinical trials can be performed only under special conditions. Therefore, due to the robust number of patients and accurate statistical analysis they have the power to change medical dogmas and the standard of care for a particular disease (Chew et al., 2011). Strict compliance and selective inclusion criteria might explain why clinical trial outcomes do not always perfectly replicate in real life. Therefore, after clinical trial completion, additional studies are usually performed on more complex patients under the current clinical practice.

1.2. New diagnostic and therapeutic techniques in ocular surface pathology

1.2.1. Introduction

The ocular surface includes the conjunctiva and the cornea, together with elements such as the lacrimal gland, lacrimal drainage apparatus, and associated eyelid structures (Knop et al., 2007). Among all these structures, the cornea is of particular interest as, due to its transparency and the refraction power of around + 40 dioptres, it is the most important dioptre of the eye (Patel et al., 2019). Thus, any corneal opacity will induce a visual impairment and will significantly decrease the quality of life. To date, around 6 million people around the world have a moderate to severe vision loss due to corneal opacities including trachoma, representing around 3% of all cases of blindness (Flaxman et al., 2017). An estimated 1.5-2 million cases of unilateral cornea-related blindness are reported every year (Whitcher et al., 2001).

Among all causes of corneal opacities, infectious keratitis (IK) is the most common one (Ung et al., 2019). There is a significantly lower incidence in developed economies (between 2.5 and 40.3 per 100.000 population-year) as compared to under-resourced countries (between 113 and 799 per 100.000 population-year) (Ting et al., 2021). In most cases, one or more predisposing factors decreasing the corneal defensive mechanisms can be identified. Ocular surface diseases (OSD), inappropriate contact lens wear, corneal trauma, or various ocular surgeries (such as cataract surgery, refractive surgery, pterygium surgery, corneal collagen cross-linking, or corneal transplantation), are frequently noticed. The risk factors and the epidemiological pattern largely vary with the demographic factors like gender, age, and socioeconomic status. In particular, the low socioeconomic status has been highly associated with IK, especially when, due to prior use of self-medication and/or traditional medicine there is a major delay in ophthalmological evaluation (Gautam et al., 2018). On the contrary, ocular surface diseases, including dry eye, blepharitis, neurotrophic keratopathy, Steven-Johnson syndrome, ocular cicatricial pemphigoid, and bullous keratopathy, have been identified as the main risk factors for IK in both developed and developing countries (Ting et al., 2021). The ocular cicatricial pemphigoid is a particular form of mucous membrane pemphigoid and is characterized by chronic bilateral conjunctivitis with relapsing-remitting periods. Without therapy, 75% of the cases will develop visual loss due to major ocular complications. Major improvements in therapy have been achieved recently.

This direction of research is reflected in the following published article:

Branisteanu DC, Stoleriu G, Branisteanu DE, Boda D, Branisteanu CI, Maranduca MA, Moraru A, Stanca HT, Zemba M, Balta F, Balta F, et al: Ocular cicatricial pemphigoid (Review). Exp Ther Med 20: 3379-3382, 2020 **IF 1.785**

https://doi.org/10.3892/etm.2020.8972

A large variety of pathogens are involved in IK including bacteria, fungi, protozoa, and viruses. While bacterial keratitis is by far the most common form in North America, Europe, Australia, and Oceania, in other parts of the world, especially in tropical climates, fungal causes are equal or greater in frequency compared with bacterial ones (Ung et al., 2019). As microbial keratitis is a medical emergency the timely beginning of appropriate therapy is mandatory to increase the chances of recovery and of preserving visual acuity. Empirical treatment with broad-spectrum anti-microbial medication is a frequent practice, provides favorable results in most cases, and is supported by the finding that 96% of isolated microorganisms are sensitive to empirical therapy (McLeod et al., 1996). When therapy fails as a result of increased resistance of microorganisms to empirical therapy the identification becomes mandatory. The identification of the causative agent by clinical examination only is not accurate in a great number of cases. Although smears and cultures obtained from corneal scrapes are considered the gold standard in making the diagnosis, these methods are far from ideal, as their sensibility is lower than desired (Ung et al., 2019). In recent years, newer techniques have been developed and improved to aid the correct diagnosis. Despite the presence of consistent literature on this topic, there is no guideline to date to have a much clearer image of each technique-specific indication in the current diagnosis armamentarium.

This direction of research is reflected in the following published article:

Zemba M, Dumitrescu O, Dimirache A, **Branisteanu DC**, Balta F, Burcea M, Moraru AD and Gradinaru S: Diagnostic methods for the etiological assessment of infectious corneal pathology (Review). Exp Ther Med 23: 137, 2022 **IF 2.447**

https://doi.org/10.3892/etm.2021.11060

Besides medical treatment, modern options for treatment and recovery after IK were recently reported. Among them, conjunctival flap surgery, collagen cross-linking (CXL), and amniotic membrane transplantation are of major interest.

Conjunctival flap (CF) surgery's main purpose is to recover the integrity of the corneal surface to prevent gradual corneal ulceration and secondary infection, as well as to achieve several other secondary effects like relieving pain, reducing topical medications, enhancing the aesthetic appearance and providing an option to invasive surgery or enucleation (Lim et al., 2009). Although it has shown a decrease in interest in developed countries after the emergence of therapeutic penetrating keratoplasty, amniotic membrane transplantation and epithelial transplantation techniques it should be taken into account in selected cases. The therapeutic effects of CF surgery are probably promoted by several mechanisms that include the mechanical covering of the affected corneal tissue (thus preventing tears, proteolytic enzymes, and proinflammatory mediators from reaching the corneal ulcer and causing stromal lysis) (Jhanji et al., 2011), supplying the compromised corneal tissue with vascularized tissue that defends the cornea from more damage (Abdulhalim et al., 2015), relieving pain and corneal sensitivity. Many of the original indications defined for this procedure (including herpetic keratitis, bullous keratopathy, neuroparalytic keratopathy, and traumatic relapsing keratopathy) are treated at present with newer, more efficient medical or surgical therapies. CFs is usually not successful in cases of active refractory bacterial and fungal corneal ulcers. However, in emergencies, especially in countries like ours requiring more cornea donors, CF surgery can still be employed as a temporary measure to inhibit disease progression and to maintain globe integrity, until a secondary surgical intervention can be performed.

This direction of research is reflected in the following published article:

Zemba M, Stamate A, Tataru CP, **Branisteanu DC** and Balta F: Conjunctival flap surgery in the management of ocular surface disease (Review). Exp Ther Med 20: 3412-3416, 2020 **IF 1.785**

https://doi.org/10.3892/etm.2020.8964

Collagen cross-linking (CXL) is a treatment option for corneal ectasia and its application has been recently extended to corneal melting and infectious keratitis. CXL could be a treatment perspective for infectious keratitis resistant to antimicrobial therapy or associated with corneal melting as ultraviolet light and riboflavin have a direct antimicrobial effect and increase the mechanical strength of the cornea (Chan et al., 2018). Recent results suggested the possible promising action of CXL for the treatment of infectious keratitis. CXL is thought to have multiple mechanisms of action on the corneal tissue, strengthening the mechanical properties of the cornea, increasing tissue resistance to enzymatic digestion, promoting healing and reducing inflammation, relieving pain, and restoring the corneal architecture (Panda et al., 2012; Bamdad et al., 2015; Shetty et al., 2014). As this protocol is time-consuming, important efforts have been made to decrease the total procedure time. Thus, the so-called 'accelerated protocols' (A-CXL) were developed and the concept of photoactivated chromophore for infectious keratitis (PACK-CXL) was created to better distinguish the use of CXL for the treatment of infectious keratitis from CXL for the treatment of progressive keratoconus (Ting et al., 2019).

This direction of research is reflected in the following published article:

Barac I, Balta G, Zemba M, Branduse L, Mehedintu C, Burcea M, Barac D, **Branisteanu D** and Balta F: Accelerated vs. conventional collagen cross-linking for infectious keratitis. Exp Ther Med 21: 285, 2021 **IF 1.785**

https://doi.org/10.3892/etm.2021.9716

The amniotic membrane is the innermost layer of the placenta, consisting of a single layer of metabolically active epithelium, a thick basement membrane, and an avascular stromal matrix. During transplantation, it exhibits various biological properties, including wound healing, anti-inflammatory, antimicrobial, and anti-angiogenic properties. The lack of graft rejection and the improvement in storage methods have awarded amniotic membrane transplantation (AMT) a particular place in the treatment of various ocular surface diseases. To date, although in small case series, many studies have evaluated the benefit of AMT for treating active IK.

This direction of research is reflected in the following published article:

Stanca TH, Balta F, **Branisteanu D**, Munteanu M. The amniotic membrane - why and how to use it in ophthalmology, Therapeutics Pharmacology and Clinical Toxicology, 2013, 17(1): 27-30 (Index Copernicus)

1.2.2. Aim

In **ocular cicatricial pemphigoid, etiological assessment in infectious corneal pathology, conjunctival flap surgery, and the amniotic membrane transplantation**

the studies aimed to realize an updated review, according to the most relevant, reliable, and recent literature data.

In the case of **cross-linking for infectious keratitis,** the aim was to evaluate, through a prospective, comparative study, the efficacy and the safety of one of the newest therapeutic methods in IK, the accelerated corneal collagen CXL.

1.2.3. Material and methods

For the reviews, the authors performed an extensive literature search in the Medline electronic database, using the PubMed interface. The search process comprised mainly articles written in English, published in the last 5 years, or more if the resulted data was limited. Different keyword combinations, adding to the pathology name words like "diagnosis" or "treatment" for example were used to refine the search. The title and abstract were subsequently evaluated and the most relevant studies or previous reviews were retained. Studies of animal models and letters to the editors, editorials, comments, and conference presentations were excluded.

Accelerated vs. conventional collagen cross-linking for infectious keratitis was designed as a prospective, comparative study. It included adult patients with a chronic corneal ulcer of microbial or fungal etiology, refractory to local and systemic etiologic treatment. After a complete ophthalmological evaluation patients were divided into two groups. Group A underwent conventional corneal Collagen CXL (PACK-CXL), using Dresden protocol and group B underwent accelerated corneal collagen CXL (PACKA-CXL). Direct samplings of the infected corneal ulcer and lower conjunctival fornix were submitted to the central laboratory for culture and antibiogram. Patients were followed postoperatively and at 3 days, 1 week, 2 weeks, 1 month, 2 months, 3 months, 6 months, and 12 months. The systemic treatment consisted of broad-spectrum new-generation antibiotics and antifungal drugs. The local treatment included fortified antibiotic and antifungal drops and also atropine. The differences between the 2 groups in final visual acuities and healing time were studied, comparing on one side the mean of quantitative variables using the t-test and on the other side the frequencies of qualitative variables using the Fisher exact test.

1.2.4. Results

The **Ocular cicatricial pemphigoid** (OCP) is nowadays considered a subtype of mucous membrane pemphigoid characterized by chronic bilateral conjunctivitis with relapsing-remitting periods (Arafat et al., 2014). The incidence rates largely vary from 1 per 10,000 to 60,000, with no racial predilection and women twice as affected as men. (Dacosta et al., 2012). Systemic practolol therapy (used in cardiac arrhythmias), topical antivirals (e.g. idoxuridine), and antiglaucomatous drugs (e.g. pilocarpine, timolol, epinephrine, parasympathomimetic derivatives, phospholine iodide) have been identified as triggers of OCP. If untreated, 75% of the cases will develop visual loss due to major corneal and conjunctival complications (dry-eye syndrome, corneal erosions, corneal keratinization, entropion, symblepharon).

The OCP pathogenesis remains unclear, with strong evidence for a type II hypersensitivity response involving circulating autoantibodies against different subunits of integrin and laminin that are mainly located in the hemidesmosome-epithelial membrane complex of the conjunctival and squamous epithelium (Chan et al., 1999). The autoantibody-autoantigen conflict activates numerous inflammatory mediators [interleukin-1 (IL-1), IL-13, tumor necrosis factor-α (TNF-α), migration inhibition factor,

macrophage colony-stimulating factor] and proteolytic enzymes responsible for the separation of the epithelium from the basement membrane with subsequent bullae formation (Saw et al., 2011). The migration of lymphocytes, eosinophils, neutrophils, and mast cells into the *substantia propria* is responsible for chronic conjunctivitis manifestations. Massive neutrophilic infiltrate of the lacrimal gland results in elevated levels of IL-8, matrix metalloproteinase (MMP)-8 and -9, and myeloperoxidase (MPO) (Chan et al., 2011). Persistent epithelial defects and stromal ulcerations due to entropion and trichiasis are major risk factors for IK. Limbal stem cell failure leads to complete corneal keratinization with neovascularization. Symblepharon and ankyloblepharon mutilate the fornices and also limit ocular movements. A genetic predisposition was revealed due to the presence of human leukocyte antigen DR2 (HLA-DR2), DR4 (HLA-DR4 [HLA-DR*0401]), and DQw7 (HLA-DQw7 [DQB1*0301]) genotypes (Xu et al., 2013).

Ocular involvement in OCP is the second most frequent (61% of the cases) after oral involvement (90% of the cases). Although the diagnosis is mainly based on ocular clinical findings, conjunctival biopsy with direct immunofluorescence remains the gold standard in OCP diagnosis confirmation. A typical positive result will reveal in the epithelial basement membrane a linear deposition of different immunoglobulins (IgG, IgA, IgM) and complement 3 proteins. Still, 20-40% of patients have a negative biopsy result that does not rule out the diagnosis (Labowsky et al., 2017). Radioimmunoassay and immunoblot techniques can identify the circulating autoantibodies in active disease while classic indirect immunofluorescence is not helpful. Despite several clinical scoring systems for OCP (Foster, Mondino, and Brown), there is still no consensus regarding which system clinicians should use. Mondino and Brown's Classification System is based on inferior forniceal depth loss (Mondino et al., 1981) while Foster's Classification System is based on clinical findings (Elder et al., 1996). The differential diagnosis mainly includes atopy, allergies, trauma, chemical burns, radiation, and neoplasia. Pseudopemphigoid medicamentosa is a common similar clinical finding with a drug-related origin (eg. pilocarpine, epinephrine, timolol, idoxuridine, echothiophate iodide, and demecarium bromide).

With long-term systemic therapy, 90% of the cases can be efficiently controlled nowadays, and only 10% of the cases will progress, as compared to 75% of the cases progressing without treatment. In mild disease and patients without glucose-6-phosphate dehydrogenase (G6PD) deficiency, Dapsone is the first-line agent, with special care for secondary hemolysis and methemoglobinemia. Oral sulfasalazine or sulfapyridine might represent an option in cases not suitable for Dapsone. If symptoms are not controlled within 3 months, systemic azathioprine or low-dose methotrexate has to be considered (Neff et al., 2008). In moderate disease, immunomodulatory/immunosuppressive therapy is initiated simultaneously with corticosteroids that allow a quicker and better control of acute phases of severe or rapidly progressive disease. In patients without thiopurine methyltransferase (TPMT) deficiency, azathioprine is a valid alternative to treatment. The Systemic Immunosuppressive Therapy for Eye Diseases Cohort Study (SITE), one of the largest retrospective cohort studies of patients with non-infectious ocular inflammatory diseases, showed that cyclophosphamide was effective in 70.7% of patients with OCP in controlling inflammation at 1 year, with 66.9% patients on low doses of prednisone (≤10 mg) (Williams et al., 2011). Mycophenolate mofetil has proved effective and well-tolerated while cyclosporine effectiveness has been reported as variable. Tetracyclines are well-tolerated and effective in mild to moderate OCP, especially combined with nicotinamide (Kempen et al., 2008). In severe disease, orally or intravenously cyclophosphamide is the first line of treatment. Adding steroids will allow a more rapid

control. Intravenous Immunoglobulin (IVIG) administration has also been found effective in severe disease, but due to significant systemic complications (anaphylaxis, disseminated intravascular coagulation, acute renal failure), it is reserved for refractory cases. Anti-TNF agents etanercept and infliximab, IL-2 antagonist daclizumab, and the anti-CD20 antibody rituximab (alone or in combination with IVIG) also proved efficacy in patients with refractory OCP (Foster et al., 2010). Topical therapy is usually for life and it is an important adjunct but never a substitute for systemic treatment. Long-term proper lubrication of the ocular surface can be achieved with preservative-free artificial tears, lubricating ointments, and silicone punctal plugs. Advanced formulations restore moisture and also prevent fluid loss. Severe keratitis not improved by tear substitutes or autologous serum drops benefits from long-term administration of topical cyclosporine-A and tacrolimus. Oral doxycycline helps control local inflammation. Topical and subconjunctival steroids can be used in the short term only to relieve symptoms. Qualitative tear testing has shown promising results in OCP. Membrane array analysis of tear proteins better quantifies the efficacy of systemic immune therapy efficacy when IL-8 and MMP-9 values decrease. Surgery of complications is a difficult task as minor conjunctival trauma can worsen the disease. As a general rule, it is recommended the case should be deferred, if possible until the disease is quiescent. In particular, surgery for entropion, symblepharon, or ankyloblepharon carries a higher risk for exacerbation. Amniotic membrane transplantation and mucous membrane grafting are used to reconstruct the conjunctival fornices. Cryotherapy rather than mechanical epilation is preferred as a treatment of trichiasis. Sutureless amniotic membrane grafting provides growth factors and anti-inflammatory cytokines, similar to autologous serum drops that promote epithelial healing. Cataract surgery has a better prognosis than glaucoma surgery due to the minimally invasive technique based on clear corneal small incisions. A keratoprosthesis or osteo-odonto-keratoprosthesis might represent the last chance for maintaining vision in severe end-stage disease with massive corneal keratinization as corneal transplantation has a very poor prognosis in such cases. The use of systemic corticosteroids perioperative can lower the risk of iatrogenic exacerbation.

Regarding the review of the **diagnostic methods for the etiological assessment of infectious corneal pathology,** 4 main diagnostic methods were identified during the research:

1. *Corneal scraping, smears, and cultures*

Smears and cultures are the conventional diagnostic methods and are indicated when the corneal infiltrate is large, central, and/or associated with significant stromal involvement. Also, if the infection is chronic or unresponsive to broad-spectrum antibiotic therapy or the clinical features are suggestive of non-bacterial keratitis (Lin et al., 2018). Ideally, sample collection should take place before any antimicrobial therapy is commenced, as isolation rates are lower in pre-treated cases. After unsuccessful initial therapy antimicrobial therapy is halted 24 h before sampling, to increase the chance of microorganism recovery (Carnt et al., 2017).

The ‘corneal scrape’ is performed from the leading edges and the base of the ulcer under topical anesthesia with a surgical blade or spatula. The study of Benson and Lanier revealed the superiority of calcium alginate swabs moistened in soy trypticase broth over platinum spatulas at yielding positive cultures and recommended using both methods, scraping with the spatula first and then rubbing the ulcer bed with the calcium alginate swab. Other sampling methods that have yielded favorable results are the ‘Mini-tip Culturette’ (Epley et al., 1998) and the corneal impression membrane, the latter used in

conjunction with transport media and further subculturing in a microbiology laboratory (Kaye et al., 2016).

Smears are obtained by spreading scraped material over clean glass microscope slides, followed by fixation and staining. Standard stains include Gram and Giemsa stains and 10% potassium hydroxide (KOH) with or without a calcofluor white stain. The Gram stain allows distinction between Gram-positive (which appear purple) and Gram-negative (which appear pink) organisms, while also revealing their shape, grouping, and relation to other components of the smear. KOH smears appear to be superior to Gram stains in their capability of displaying fungi, Nocardia spp. and Acanthamoeba spp. (Bharathi et al., 2006). On the KOH smears, as cellular debris is cleared, the refractile hyphal fragments of fungi are readily observed. In Gram-stained smears, Nocardia appears as Gram-positive, beaded, thin branching filaments, while in KOH smears, Nocardia can be recognized as very fine, narrow, intertwined, branching filaments, more easily identifiable. The KOH wet-mounted stain also displays the characteristic double-walled cysts of Acanthamoeba, which are not always apparent in Gram stains or can be confused with other components of the smear, such as inflammatory cells. Specificities of both the Gram and the KOH smears were over 83% in most studies (Shimizu et al., 2020). If KOH + calcofluor white smears are positive for fungi, antifungal therapy should be promptly initiated and, if the KOH + calcofluor white smear is negative for fungi and Acanthamoeba spp., broad-spectrum antibiotics should be the treatment of choice (Sharma et al., 2002). A synopsis of the sensitivities and specificities reported by different studies of the Gram and KOH stains are presented in Table 1.1.

Table 1.1 - Sensitivity and specificity of the Gram and the KOH stains in the diagnosis of infectious keratitis.

Study	Number of eyes investigated	Microorganism	Gram		KOH	
			Sn	Sp	Sn	Sp
Bharathi *et al* (18)	3298	Fungi	89.2%	100%	99%	99.1%
		Bacteria	100%	96.7%	NA	NA
		Nocardia spp.	87%	97.6%	100%	100%
		Acanthamoeba spp.	60%	100%	91.4%	100%
		Overall (bacteria + fungi + *Acanthamoeba* spp.)	93.8%	93.9%	99.1%	91%
Eleinen *et al* (22)	88	Bacteria	33.3%	100%	NA	NA
		Fungi	NA	NA	65.9%	100%
Sharma *et al* (21)	251	Bacteria				
		Fungi	36%	84.9%	NA	NA
					61.1%	99%
Sharma *et al* (21)	841	Bacteria	40.9%	87.1%	NA	NA
		Fungi	NA	NA	87.7%	83.7%
Badiee *et al* (29)	38	Fungi	21%	100%	68%	100%
Panda *et al* (8)	122	Bacteria	45.25%	92.75%	NA	NA
Shimizu *et al* (23)	272	Bacteria	63.1%	89.8%	NA	NA

Sn, sensitivity; Sp, specificity; NA, not assessed.

The main factors influencing the results are the ulcer dimensions, the amount of scraped material, the accuracy of microscopic examination, and previous antimicrobial therapy (Sharma et al., 2002). It was suggested that smear examination is more relevant for therapy than cultures, as the results are more readily available. However, the gold

standard of diagnosis and the confirmation of smear results reside in cultures (Das et al., 2010). Aside from providing certainty regarding the etiology of the keratitis, cultures are the only method that allows testing for antibiotic sensitivity (Lin et al., 2019).

Cultures are obtained by inoculating the scraped material onto solid or liquid media. After sampling, scraped material is transferred to agar plates (the direct method), or is sent to a microbiology laboratory with the help of a liquid transport medium, where further subculturing ensues (indirect method) (Kaye et al., 2003). Inoculation is conducted at the surface of solid media or by direct swirl into liquid media. Typical solid media include sheep or horse blood agar (BA), chocolate agar (CA) which contains nutrients for the growth of fastidious microorganisms (14), Sabouraud's dextrose agar (SDA) with antibiotics (chloramphenicol and/or gentamycin) for the culture of fungi, non-nutrient agar (NNA) with Escherichia coli (E. coli) overlay for Acanthamoeba spp. Special media, such as Löwenstein-Jensen for mycobacteria, and media for anaerobic microorganisms can be used in selected cases.

A study by Das et al., in 2010, revealed the non-inferiority of BA and CA compared with SDA in the detection of fungi. Bhadange et al., in 2013, found that liquid media are adjuvant to solid media, aiding in the diagnosis of bacterial and mixed infections, and suggested that they should be included in standard diagnostic protocols. Kaye et al., in 2013, found no significant difference between microbial isolation rates with the direct and indirect methods. The study of McLeod et al concluded three possible scenarios in real-life practice, In the first scenario, the safest but the most expensive one, standard smears, and cultures are performed in all cases, with the main aim of identifying non-bacterial microorganisms and starting appropriate treatment early. The second scenario proposes microbiological assessment only in cases where the history or clinical examination raises the suspicion of a non-bacterial or severe infection (a severe ulcer was defined as one that encompassed the visual axis or involved more than half of the cornea or resulted in corneal thinning to less than half of the normal thickness or was associated with hypopyon). All other cases should receive treatment with broad-spectrum, fortified antibiotics, with close follow-up. According to the third scenario, practitioners should scrape and directly culture all corneal ulcers, and then the inoculated plates should be kept in proper conditions in a microbiology laboratory and only be examined if the initial therapy fails. If the initial treatment proves effective, the plates can be discarded without further analysis.

2. *Corneal biopsy and histopathological examination*

The corneal biopsy is a valuable tool if there is a failure of the initial therapy but carries the risks of corneal perforation, scarring, or irregular astigmatism with a significant effect on the final visual outcome. The corneal biopsy provides larger and more profound tissue than corneal scrapes. The histopathological examination has the advantage of a quicker response (often within 72 h), being of great value in identifying organisms that are either difficult or slow to culture. Before the biopsy, antimicrobial therapy should be withheld for at least 24 h (Robaei et al., 2018). The procedure may be performed at the slit lamp, in a minor procedure room, or the operating room under topical anesthesia. If possible, the visual axis should be avoided. The biopsy specimen is placed in a balanced salt solution and sent to the microbiology laboratory, where it undergoes homogenization with sterile trypticase soy broth and the mixture is then inoculated into the appropriate media. If a histopathological examination is desired, the specimen can be bisected, with one half being placed in 10% buffered formalin and sent to a pathology laboratory. For the histopathological examination, the material is processed for paraffin embedment, cut into 3-mm (16) or 5-mm slices with the microtome, and then stained. Routine stains include hematoxylin-eosin (H&E), Gram

stain for bacteria, Gomori methenamine silver (MethAg), periodic acid Schiff (PAS) for fungi, Ziehl Neelsen, Fite special stain, and the auramine fluorescent stain for mycobacteria (Robaei et al., 2018). The biopsy positivity largely varies in the literature between 42% (Younger et al., 2012) to 82% (Alexandrakis et al., 2000).

3. *Molecular assays: Polymerase chain reaction (PCR) and next-generation sequencing (NGS)*

PCR is a sensitive and rapid molecular method used to amplify and render detectable minute quantities of microbial DNA in pathological specimens. PCR has been successfully used in the diagnosis of uveitis, endophthalmitis, as well as of viral ocular infections (Shimizu et al., 2020). Corneal material for the PCR assay is usually obtained by corneal scraping. DNA extraction is performed to separate DNA from proteins, cell membranes, and components. This can be performed manually or using commercially available kits. After DNA extraction, a PCR mix is prepared by adding the reagents separately or by using a master mix, which contains the necessary ingredients. The reaction mixture contains the template DNA, the four nucleotides in equal proportions, two primers, one forward and one reverse, a buffer, and the Taq-DNA polymerase (Simon et al., 1991). Most investigations begin with a broad-range PCR, with primers that are either bacterium- or fungus-specific. This allows differentiation between bacterial and fungal keratitis, which is critical to therapy, as numerous clinicians are hesitant, especially when the history and the clinical aspect are not very suggestive, to initiate antifungal therapy, fearing its potential toxicity. PCR has obvious advantages that render it superior to smears and cultures in the detection of pathogens. Firstly, it provides quick results, usually within 2-8 h (compared with a minimum of 24-48 h in the case of cultures), therefore permitting the prompt choice of appropriate therapy based on the result. Secondly, the method requires a small amount of tissue, which is helpful considering the small volume of samples usually available from corneal scrapes. Most importantly, it requires very little pathological DNA, one copy of DNA being enough to detect the pathogen. Still, PCR lacks the high specificity of smears and cultures and has an important rate of false-positive results, as microorganisms belonging to ocular flora, tears, or laboratory contaminants can be amplified in the reaction and considered causative agents (Itahashi et al., 2010). PCR can detect both viable and non-viable microorganisms and this can be a disadvantage.

There are numerous variants to the classic, direct PCR. Multiplex PCR permits amplification of more than one target at a time, thus allowing analysis and identification of multiple microorganisms in the same PCR cycle. Nested PCR comprises two consecutive PCR reactions, in which the result of the first reaction is used as a target for the second reaction (Gupta et al., 2019). This technique has increased sensitivity, but should not be routinely used, as it has a high rate of false positives. Real-time PCR uses special dyes to estimate the quantity of the product in the sample as the amplification progresses (Gupta et al., 2019). Most studies report high PCR sensitivities, of over 85%, in bacterial and fungal keratitis, higher than cultures and smears, as summarized in Table 1.2.

NGS is a metagenomic assay, in which millions of small (150-500 bp in length) DNA fragments are simultaneously sequenced in a matter of hours (Ma et al., 2019). In cases of infectious keratitis, sample tissue is often represented by a corneal biopsy which is formalin-fixed paraffin-embedded. NGS is divided into two strategies: Targeted amplicon sequencing (TAS), which uses PCR as a starting point, and microbial whole-genome sequencing (MWGS), which sequences fragments found throughout the microbial genome. While TAS is more cost-effective and useful at identifying certain pathogens of interest, MWGS has the advantage of unbiased sequencing and is, thus,

valuable in detecting new or atypical pathogens. Resultant sequences are then compared with databases of microbial DNA with the help of metagenomic classification engines, such as Centrifuge and Kraken, to identify if they match any known pathogen (Li et al., 2018). The assay usually requires at least 3.5-4 days, which makes it comparable to cultures.

As compared to real-time PCR as the standard, the NGS-based assay provided favorable results, with a sensitivity of 88% and a specificity of 100%. Discrepancies between NGS and culture results may indicate a possible role for NGS as a complementary method, especially in cases unresponsive to treatment (Ren et al., 2020). The specificity and positive predictive values of PCR reported by different studies are summarized in Table 1.3.

Table 1.2 - Comparative sensitivities of smears, cultures, and PCR in different studies.

Study	Number of eyes investigated	Microorganism	PCR type	Target	Smear type	Sensitivity		
						Smear	Culture	PCR (%)
Eleinen *et al* (22)	88	Bacteria	Direct PCR	16S rRNA gene	Gram	33.33%	57.33%	87.88
Eleinen *et al* (22)	88	Fungi	Direct PCR	18S rRNA gene	KOH	65.91%	59.09%	90.91
Badiee *et al* (29)	38	Fungi	Nested PCR	18S rRNA gene	KOH Gram	68% 21%	57%	75
Panda *et al* (8)	122	Bacteria	Direct PCR	16S rRNA gene	Gram	45.25%	NA	86.96
Shimizu *et al* (23)	272	Bacteria	Real-time PCR	16S rRNA gene	Gram	63.1%	51.8%	63.6
Zhao *et al* (36)	67	Bacteria Fungi *Acanthamoeba* spp.	Direct PCR without template DNA extraction	5.8S rRNA gene 16S rRNA gene The conservede 29 region of 18S rRNA gen The US4 region of the envelope glycoprotein G	Gram + KOH	NA	47.1%	81.8

NA, not assessed.

Table 1.3 - Specificity and positive predictive values of PCR reported in different studies.

Study	Number of eyes investigated	Microorganism	PCR type	Primer target	Specificity (%)	Positive predictive value (%)
Badiee *et al* (29)	38	Fungi	Nested PCR	18S rRNA gene	70	50
Panda *et al* (8)	122	Bacteria	Direct PCR	16S rRNA gene	86.96	83.93
Shimizu *et al* (23)	272	Bacteria	Real-time PCR	16S rRNA gene	67.5	44.9
Zhao *et al* (36)	67	Bacteria Fungi *Acanthamoeba* spp.	Direct PCR without template DNA extraction	5.8S rRNA gene 16S r RNA gene The conserved 29 region of 18S rRNA gene		
				The US4 region of the envelope glycoprotein G	81.8	96.2

4. In vivo confocal microscopy (IVCM)

IVCM is a rapid, repeatable, non-invasive examination that allows real-time visualization of corneal layers and structures, as well as of potential pathological agents. Main indications for IVCM are the sterile anterior corneal stroma during current treatment with antifungal or anti-Acanthamoeba spp. therapy, deeply situated infiltrates where corneal scrapes do not have access and microbial keratitis occurring after corneal surgery, such as intracorneal implants and refractive surgery (Vaddavalli et al., 2011). IVCM is a contact assay using an immersion lens and, therefore, requires topical anesthesia, a coupling agent, and a disposable sterile cap that is applied to the ocular surface. Examination of the captured images can demonstrate, in cases of infectious keratitis, inflammatory cells and characteristic aspects of different microorganisms, especially fungi and Acanthamoeba spp. Goh et al., compared IVCM with cultures and real-time PCR (the Riviere assay) in cases of suspected Acanthamoeba keratitis and found that IVCM had the highest sensitivity of the three. The high sensitivity and specificity render IVCM a valuable adjuvant to the other diagnostic assays and some authors advocate for its use early in the course of the disease. Moreover, being non-invasive, IVCM can be repeated during the treatment, thus also being useful in monitoring the therapy (Vaddavalli et al., 2011). In cases of uncooperative patients, there is a higher rate of false-negative results. Higher false-negative results are also yielded by excessively hazy corneas or by an abundance of inflammatory cells that mask the characteristic elements of pathogens. The sensitivity and specificity of IVCM in different studies are summarized in Table 1.4.

Table 1.4 - Sensitivity and specificity of IVCM in different studies.

Study	Number of eyes investigated	Microorganism	Sensitivity (%)	Specificity (%)
Goh *et al* (49)	25	*Acanthamoeba* spp.	88.9-100	100
Kanavi *et al* (50)	133		100	84
Vaddavalli *et al* (47)	148		80	100
Wang *et al* (10)	49		91.7	100
Kanavi *et al* (50)	133	Fungi	94	78
Vaddavalli *et al* (47)	148		89.2	92.7
Wang *et al* (10)	49		66.7	100
Vaddavalli *et al* (47)	148	Fungi + *Acanthamoeba* spp.	88.3	91.1
Wang *et al* (10)	49		85.3	100
Wang *et al* (10)	49	Bacteria	66.7	100
Wang *et al* (10)	49	Viruses	100	100

During the review of **conjunctival flap surgery in the management of ocular surface disease,** some key features have emerged**.** Conjunctival flap surgery (CFs) is a well-recognized method for assuring a stable ocular surface and repressing inflammation in compromised corneas or eyes with reduced visual potential (Gundersen et al., 1969). Although its use has decreased in developed countries after the emergence of therapeutic penetrating keratoplasty, amniotic membrane transplantation, and epithelial transplantation techniques, it should be taken into consideration in selected cases. The alternative treatments constitute considerable technological progress but have limited availability in developing countries, especially the deficit of corneal donor tissue for corneal transplantation.

The main purpose of the surgery is to recover the integrity of the corneal surface to prevent gradual corneal ulceration and secondary infection, as well as to achieve

several other secondary effects such as relieving pain, reducing topical medications, enhancing the aesthetic appearance, and providing an option to invasive surgery or enucleation. The conjunctival flap covers the affected corneal tissue and prevents tears, proteolytic enzymes, and proinflammatory mediators from reaching the corneal ulcer and causing stromal lysis (Stamate et al., 2019). Also, supplies the compromised corneal tissue with vascularized tissue that defends the cornea from more damage; the rich supply of blood and lymphatic vessels provides nutrients, including cellular components and growth factors, that increase the resistance to infection and anticollagenolytic substances that suppress stromal ulceration (Sharma et al., 2014). As the original indications defined for this procedure (herpetic keratitis, bullous keratopathy, neuroparalytic keratopathy, and traumatic relapsing keratopathy), are treated at present with other medical or surgical therapies, the list of indications has expanded over time. Chronic infectious keratitis and tectonic indications such as peripheral marginal ulcerations and corneal melts can be successfully managed with the conjunctival flap (Zhou et al., 2010).

In the particular case of IK, CFs is usually not successful in cases of active refractory bacterial and fungal corneal ulcers. However, in emergencies, especially in countries that lack cornea donors, CF surgery can be employed as a temporary measure to inhibit disease progression and to maintain globe integrity, until a secondary surgical intervention can be performed for vision restoration. The recent literature confirms this indication through several published studies. Nizeyimana et al., obtained a good postoperative outcome for all their patients with refractory fungal keratitis, with or without perforation, with control of the infection and preservation of the globe. Moreover, the study performed by Zhong et al., that combined full-thickness conjunctival flap surgery with amniotic membrane transplantation in the treatment of severe fungal keratitis without perforation showed 88.24% of eyeball preservation. Abdulhalim et al., compared the results of bipedicle CF and cryopreserved amniotic membrane graft in the treatment of non-viral infectious keratitis resistant to medical treatment. Successful results were observed in 18 out of 20 eyes (90%) in each group. Their results indicated that CF and amniotic membrane transplantation are efficient as a surgical treatment for infectious keratitis because they could recover ocular surface integrity and offer metabolic and mechanical support for corneal healing. The use of an amniotic membrane can have a benefit as it does not hinder corneal examination like CF, it does not affect limbal stem cells, and supplementary, it conserves the conjunctiva which may be required for another ocular surgery.

CF surgery results can be influenced by both intraoperative and postoperative complications. Most of them can be avoided with a careful case selection, a proper preoperative evaluation, and a meticulous surgical technique. Usually, sutures that are too tight may cut through a tight CF and a loose CF may not heal and detach in the postoperative period. The most frequent postoperative complications include flap retraction, conjunctival buttonholes and erosions, epithelial inclusion cysts, corneal perforations, and ptosis (Lim et al., 2009). In particular, the persistence of infection under the flap significantly affects the stability of the ocular surface.

As regards **the amniotic membrane (AM),** and why and how to use it in ophthalmology, the following interesting results came to light. The AM is the internal layer of the fetal membrane in direct contact with the amniotic fluid and histologically is constituted by one single layer of epithelial cells, placed on a basal membrane and an avascular stroma. The amniotic cells are connected laterally by numerous desmosomes, but there are no tight junctions and the lateral intercellular space may provide an effective transcellular pathway for macromolecule transportation. Macroscopically, the amniotic

membrane is thin, elastic, semi-transparent, and adherent to the chorion, from which it detaches easily by simple hauling or with the help of a spatula. It's easy to handle and it's not so different from the conjunctiva by consistency, thickness, and sensation on palpation. It can stretch between forceps without breaking and for suture, we can use the same materials (needles and threads) as for the conjunctiva. It can be used fresh (right after sampling), conserved for a short time in fluids similar to those used for the conservation of cornea or freeze. Kept under -80° C, the amniotic membranes can be thawed at any request and transported to the surgeon in a protecting fluid medium. The medical regulations in U.S.A. and E.U. preclude using fresh amniotic membranes, despite the fact they function as well as preserved ones when transplanted onto the ocular surface because there are certain concerns, the main one being the risk of transmission of diseases (Adds et al., 2001). As part of the processing of preserved amniotic membranes, the maternal donor undergoes serologic testing at the time of the procurement of donor tissue and again 6 months later (Dua et al., 1999). The tissue is used for grafting only when both samples are negative.

In ophthalmology, the first use of the amniotic membrane dates from 1940, when De Roth used amnion and chorion to repair 6 eyes with symblepharon. Six years later Sorsby and Simons described a chemically processed amniotic membrane called *Amnioplastin* used successfully in the treatment of acute chemical ocular burns. But only in 1995, Kim, Tseng et al.,, stirred up the interest of the ophthalmologists for the amniotic membrane demonstrating experimentally that the membrane is efficient for the reconstruction of corneal surfaces after the removal of the epithelium and limbic lamellar keratectomy. The sampling is made by the obstetrician who previously selects between the cesarean candidates the ones able to donate the amniotic membrane. The amniotic membrane is harvested from the fresh placenta of the pregnant women who gave birth by cesarean (because passing through the pelvigenital connection would contaminate it), seronegative for anti-HIV, anti-HVC, anti-HVB, syphilis, CMV, toxoplasma. After harvesting, it is washed with a serum solution or BSS (Balanced Salt Solution) in which can be introduced penicillin + streptomycin + neomycin, amphotericin B. Then it is frozen at -140° C or -80° C in 1/1 glycerol-inositol solution. There are two protocols in use in the U.S.A.: one popularized by Tsubota's group wherein the membrane is cut into pieces measuring 10 cm × 10 cm and rinsed sequentially for five minutes in each of 0.5M dimethyl sulfoxide (DMSO), 1.0M DMSO, and 1.5M DMSO. The second method was popularized by Tseng and co-workers and consists of storing the pieces of the membrane in 50% glycerol in Dulbecco's modified Eagle Medium (DMEM, Gibco) or TC-199. The pieces of the membrane are usually spread epithelial side up, on nitrocellulose paper before storage in the medium. The tissue is stored frozen at -80°C and released for use only after the second serological screening test, carried out six months after delivery is normal. Tissue has been stored and used for up to 2 years post-delivery.

The amniotic membrane usage in ophthalmology has the following indications: chemical burns, uninfected corneal and sclera ulcerations and perforations, advanced cicatricial ocular pemphigoid, and Stevens-Johnson syndrome. Recently, the efficacy of cryopreserved AM as adjuvant therapy for infectious corneal ulcers (Yin et al., 2020) and also for refractory cases (Schuerch et al., 2020) was confirmed.

For the assessment of the clinical efficacy and safety of **accelerated corneal collagen CXL (PACKA-CXL) vs. conventional corneal collagen CXL (PACK-CXL) in the management of infectious keratitis,** a total number of 16 eyes from 16 patients were evaluated in a clinical study, 8 eyes randomized for each group. The mean age of the patients from Group A was 39.9±19.8 years, not significantly different (P=0.967) from

the mean age of Group B (40.2±15.0 years). Five patients from Group A and 4 patients from Group B were males, with no significant gender differences between groups (P=0.614). Five patients from Group A had a central lesion and 3 patients had a paracentral lesion; in Group B the situation was exactly the opposite (P=0.317). The patients from Group A had previous treatment for 5.2±2.6 weeks, not significantly different (P=0.738) from Group B (5.9±4.4). Seven patients from Group A and 6 from B had a positive culture, with no significant differences between groups (P=0.522).

The ulcer size (mm2) and infiltrative area (mm2) were not significantly different, but greater in Group A vs. Group B (17.6±13.5 and 29.0±21.3 vs. 17.4±15.7 and 28.0±20.1, respectively). The distribution based on superficial and deep vascularization was not significantly different between the groups (P=0.202 and P=0.289, respectively).

The mean time to healing in patients from Group A (34.9±11.4 days) was 2 days longer than in Group B (32.9±9.4 days), but the difference did not reach statistical significance (P=0.708). The demographic and clinical parameters in both groups are summarized in Table 1.5.

Table 1.5 - Demographic and clinical parameters of groups A and B.

Characteristics	Group A (PACK-CXL) (n=8)	Group B (PACKA-CXL) (n=8)	P-value
Age, years (mean ± SD)	39.9±19.8	40.2±15.0	0.967
Sex, n (%)			0.614
Male	5 (62.5)	4 (50.0)	
Female	3 (37.5)	4 (50.0)	
Site			0.317
Central	5 (62.5)	3 (37.5)	
Paracentral	3 (37.5)	5 (62.5)	
Treatment length before presentation (weeks)	5.2±2.6	5.9±4.4	0.738
Microbiologic culture			
Positive	7 (87.5)	6 (75.0)	0.522
Ulcer size (mm^2)	17.6±13.5	17.4±15.7	0.973
Infiltrate area (mm^2)	29.0±21.3	28.0±20.1	0.924
Time to healing (days)	34.9±11.4	32.9±9.4	0.708
Superficial vascularization			0.202
0	5 (62.5)	2 (25.0)	
1	1 (12.5)	3 (37.5)	
2	2 (25.0)	1 (12.5)	
3	0 (0.0)	2 (25.0)	
Deep vascularization			0.289
0	5 (62.5)	2 (25.0)	
1	2 (25.0)	3 (37.5)	
2	1 (12.5)	3 (37.5)	

PACK-CXL, conventional corneal collagen cross-linking; PACKA-CXL, accelerated corneal collagen cross-linking.

1.2.5. Discussions

In the case of the **ocular cicatricial pemphigoid,** the literature research reveals that the pathogenesis remains uncertain and probably linked to an autoimmune type II hypersensitivity response in patients with a genetic predisposition and exposure to different environmental triggers. Conjunctival biopsy with direct immunofluorescence is the gold standard in diagnosis confirmation, but up to 40% of the patients have a negative biopsy result that does not rule out the diagnosis. The skin and many other mucous membranes (e.g. oral, trachea, esophagus, pharynx, larynx, urethra, vagina, and anus)

may be involved. The disease grading mainly relies on the Foster staging system (based on clinical signs) and Mondino and Brown system (based on the inferior fornix depth loss). The differential diagnosis must include atopy, allergies, trauma, chemical burns, radiation, neoplasia, infectious, inflammatory, and autoimmune etiologies. The main goals of the treatment in OCP are to stop disease progression, relieve symptoms, and prevent complications.

Concerning the review on the **etiological evaluation of corneal ulcers,** the literature data suggests that significant improvements have been realized in the latest years. Despite being the gold standard and having good specificity, the sensitivity of smears and cultures in IK continues to be far from ideal. Culture sensitivities range from 32.7 to 79.4%. Moreover, cultures may take several days until the results are ready. This delays appropriate therapy in a condition where time is vision. Smears sensitivities lie in the interval 27.3-61.6% (Ung et al., 2008). Possible explanations for the low sensitivity of smears and cultures may reside in the previous antibiotic treatment, the small size of the samples, and the difficulty in optimally incubating the plates (Kaye et al., 2003). This explains the ongoing search for an optimized method with higher sensitivity along with a lower rate of false-positive results. The main current criteria for culture positivity include a confluent growth at the inoculation site on solid-phase media, the growth of the same microorganism on more than one solid-phase medium, consistency between culture and microscopy findings, and repeated isolation of the same microorganism after different scrapings (Robaei et al., 2018).

When used correctly, the corneal biopsy is a valuable investigation that can identify a new pathogen, not detected by previously used diagnostic methods, and lead to a case-saving change in therapy. It should, however, be used cautiously, due to its potential risks. Corneal biopsy and histopathological examination also allow, in cases with less typical clinical aspects, to exclude ocular involvement from other systemic diseases such as Lyell syndrome, Stevens-Johnson syndrome, lichen planus, or Rowell syndrome.

PCR as a sole investigation did not demonstrate significant superiority to cultures and smears, with a reported sensitivity of 63.6%. However, when used alongside cultures and smears, PCR significantly improved diagnostic efficacy. PCR and culture results were concordant in most cases. Furthermore, in culture-negative cases, PCR also yielded a smaller number of copies. Concordance rates of PCR and culture results vary widely, ranging from 43 to 93% (Itahashi et al., 2010). The high rate of false positives is perhaps the most important reason why most authors, while acknowledging the usefulness of PCR as an adjuvant in the diagnosis, still consider cultures to be the gold standard in providing the diagnosis of infectious corneal ulcers. New advances in PCR assessment aim to increase sensitivity, and specificity and help differentiate between different microorganisms. PCR is a valuable addition to smears and cultures in the diagnosis of infectious keratitis. However, to become a comprehensive diagnostic method that could replace the conventional approaches, it still requires improvements to increase specificity, reduce the rate of false positives, establish protocols and diagnostic thresholds, increase availability and reduce the costs.

Discrepancies between NGS and culture results may indicate a possible role for NGS as a complementary method, especially in cases unresponsive to treatment.

IVCM has demonstrated a need for experienced users and in conjunction with its high cost, it results in low availability. Therefore, to date, IVCM cannot be used alone, but alongside cultures and smears.

The reviews on **conjunctival flap surgery** and **amniotic membrane usage in ophthalmology** have identified new indications and a variety of improvements to the original technique over time, to overcome disadvantages and enhance the success. To date, there are 4 main types of CFs. Total conjunctival flap, originally described by Gundersen, is the most commonly used CF and is a thin, bipedicle, bridge flap that involves a 360-degree peritomy, debridement of the entire corneal epithelium, and mobilization of the conjunctiva at the superior fornix to cover the entire corneal surface. Bipedicle bridge flap (bucket-handle) is used for small central or paracentral corneal lesions that do not need coverage of the entire cornea and involves a 180-degree peritomy, separation of the conjunctiva from the underlying Tenon's capsule, an incision parallel to the limbus and mobilization of the conjunctiva over the corneal ulcer. A single pedicle flap (racquet flap) is a smaller racquet-shaped perilimbal CF that is mobilized to cover perilimbal corneal lesions. An advancement flap is used for paralimbal lesions, usually in conjunction with a lamellar corneal patch or scleral patch graft, and involves performing a peritomy and pulling the adjacent conjunctiva over the peripheral corneal lesion. Each technique has specific advantages and limits. Recently, Sandinha et al., described a different CF technique referred to as superior forniceal conjunctival advancement pedicle (SFCAP) for managing corneal perforations or impending corneal perforations. The technique implies the creation of a pedicle, which includes a prominent blood vessel, by making two parallel conjunctival incisions, placing its advancing edge on the cornea, and suturing it with 10-0 nylon interrupted sutures around the corneal ulcer. The advantages of this technique are a lesser extent of conjunctival dissection, which does not affect the local conjunctiva in case of later glaucoma surgery, and rapid healing that eliminates the stimulus for vasogenic substances and prevents the formation of neovessels in the peripheral corneal which is advantageous for future corneal grafts. Another new approach was described by Sharma et al., in which the CF was repositioned at its original site, in a patient that did not show any improvement in symptoms after the initial surgery for refractory fungal corneal ulcer. Early repositioning of the CF after healing of the cornea is beneficial as the prolonged presence of the CF on the cornea increases the risk of ocular surface damage. CF promotes corneal vascularization and the surgery itself can induce limbal stem cell loss that may lead to an unstable ocular surface, which may necessitate limbal autograft surgery. Adding cryotherapy to flap surgery may have a cumulative effect. Cryotherapy of the corneal surface at temperatures of -50 to -60˚C not only denatures the pathogen's cell walls but can also remove part of the antigen-antibody complexes and decrease inflammation caused by the area of ulceration and neovascularization (Lu et al., 2016).

The benefits of amniotic membrane transplantation on the ocular surface in various diseases can be explained by the tissue properties themselves. First, the amniotic membrane is a non-immunogenic tissue, so the transplantation does not require immunosuppressive therapy and the risk of rejection is absent. It provides antiadhesive, bacteriostatic, and antiphlogistic activity and also reduces angiogenesis. The inhibitory effect on fibroblast activity is lowering the risk for scarring and collagen destruction. Moreover, it promotes both in vivo and in vitro tissue re-epithelialization (Liu et al., 2019). In surgical applications of the human amniotic membrane to the ocular surface, the transplanted amniotic membrane is known to facilitate ocular surface healing with minimal inflammation and scarring.

In one study, the self-retained cryopreserved AM was successfully used as adjuvant therapy for infectious corneal ulcers (Yin et al., 2020). Patients receiving additional placement of cryopreserved AM showed significantly faster epithelialization and achieved complete epithelialization in significantly more cases. Also, as compared to

the control group at the time of complete epithelialization the final visual acuity was significantly better. Ting et al., performed and published in 2021 a systematic review and meta-analysis of the AM transplantation for infectious keratitis. In the evaluation, all clinical studies, including randomized controlled trials (RCTs), non-randomized controlled studies, and case series (n > 5), were included. The main parameters followed were the time to complete corneal healing, the best final visual acuity, the corneal vascularization, and the adverse events. The adjuvant AM transplantation resulted in a shorter mean time to complete corneal healing as compared to standard antimicrobial treatment alone, better final visual acuity, and no significant difference in the risk of adverse events. Also, less corneal vascularization at 6 months was noticed in the AM group as compared to standard antimicrobial treatment alone.

Referring to **corneal cross-linking**, the abundant literature data indicates that this technique represents an intensely studied method of treatment as many authors have published their results on this subject. Richoz et al inoculated ex vivo porcine corneas with a suspension of Gram-negative (Pseudomonas aeruginosa) and Gram-positive (Staphylococcus aureus) bacterial strains. After incubation, PACK-CXL was performed, at two irradiation settings: 5 min at 18 mW/cm2 and 2.5 min at 36 mW/cm2. There were significant differences ($P<0.001$) between control corneas and cross-linked corneas. The results revealed similar killing rates for both bacterial strains (approximately 93%), whether they undertook the accelerated or long protocol. The authors concluded that PACK-CXL, when used for treating infectious keratitis, seems to preserve its antimicrobial efficacy, even with a shorter time of exposure. After clinical validation, the results can be transferred to routine practice, allowing a shortened treatment time. Tabibian et al reported the case of a 24-year-old man with fungal keratitis related to contact lens wearing. Accelerated PACK-CXL as a single treatment was performed and evolution of the infiltrate was favorable within 24 h. Makdoumi et al published promising results in 16 patients who underwent PACK-CXL as first-line treatment for infectious keratitis. In a prospective study involving 40 patients, 21 treated with PACK-CXL and 19 treated with antimicrobial therapy, Said et al found that the average healing time was shorter in the PACK-CXL group, although not statistically significant. In addition, the number of related complications was lower in the PACK-CXL group, supporting the efficacy and safety of PACK-CXL in treating microbial keratitis. Rapuano et al recently described the implications of sodium hydroxymethylglycinate (SMG) in pharmacologic cross-linking of the cornea in the treatment of keratitis. The modified crosslinking (M-CXL) performed by Kasparova et al., consisted of associating the crosslinking procedure with frequent instillations of antimicrobial drops. In a group of 24 treated patients, they reported a reduction in treatment time by 42%, as compared to the control group treated with conservative treatment. Ting et al performed a comprehensive meta-analysis comprising 46 articles and 435 patients. This meta-analysis compared the standard antimicrobial treatment alone for infectious keratitis with PACK-CXL as adjuvant therapy. The authors concluded that the average healing time was less than 7 days for patients treated with adjuvant PACK-CXL when compared to 10 days for standard antimicrobial treatment alone.

1.2.6. Conclusions

The systematic review of the latest, more relevant literature data on these topics suggests the following conclusions.

Ocular cicatricial pemphigoid remains one of the most invalidating pathologies due to chronic bilateral conjunctivitis with relapsing-remitting periods. Conjunctival biopsy with direct immunofluorescence is considered the gold standard in diagnosis confirmation. To date, long-term systemic therapy can efficiently control 90% of the OCP cases. While Dapsone is the first-line treatment in mild to moderate disease in patients without G6PD deficiency, more severe cases require immunosuppressant therapy with azathioprine, mycophenolate mofetil, methotrexate, or cyclosporine. Cyclophosphamide, biologics (etanercept or rituximab), and intravenous immunoglobulin therapy are usually reserved for recalcitrant disease and unsatisfactory results to conventional therapy. Dry eye syndrome requires constant lubricating medication and topical steroids, cyclosporine-A, and tacrolimus. Surgery should be planned only in the quiescent phase as minor conjunctival trauma can significantly worsen the disease.

Concerning the **diagnostic tools for infectious corneal ulcers,** we have seen in recent years the development of numerous new techniques. Still, the gold standard for making the diagnosis resides in cultures. Cultures are highly specific and allow testing for antibiotic susceptibility. Even though cultures require a longer time than other methods until results are ready and their sensitivity is lower than desired, they still provide the diagnosis confirmation and the reference standard for the evaluation of all the other methods. If positive, smears can facilitate diagnosis and allow prompt initiation of appropriate therapy. IVCM, when accessible, is a non-invasive, rapid tool, with high specificity and sensitivity, and of great value, especially in cases of fungal and Acanthamoeba keratitis. Molecular methods, consisting of PCR and NGS, are rapidly evolving and improving and are of particular use in culture-negative cases, as well as in infections with atypical or novel organisms. They are highly sensitive but yield a high rate of false positives. Advances in technology are promising, as an improved understanding of both the commensal and the pathological organisms found on the ocular surface, as well as their interactions with each other and with the human host, is observed. In time, as molecular methods gain promptness, specificity, and sensitivity, the replacement of conventional methods with these new, improved assays may be observed.

The review on **corneal flap surgery** revealed that it is a simple, efficient, and cost-effective method of treatment for ocular surface disease resistant to medical therapy. It also relieves pain, reduces the need for frequent medications, enhances aesthetic appearance, and provides an alternative to invasive surgery or enucleation. In some instances, it can be used as a method of temporizing future corneal transplantation. CFs are underused today because of the availability of alternative therapeutic options like bandage contact lenses, amniotic membrane transplantation, epithelial transplantation, and therapeutic keratoplasty, which also, do not lack specific complications. Considering its low complication rate and its proven immediate and sustained outcome, CF surgery should be considered nowadays mainly in the management of resistant ocular surface disease.

As for the **amniotic membrane usage in ophthalmology,** the sutureless transplantation may be an effective adjuvant therapy in treating sight-threatening infectious corneal ulcers by promoting faster corneal epithelialization, less corneal vascularization, and overall better recovery of the VA. Also, it is a valuable treatment option to achieve corneal epithelial wound healing in cases refractory to conventional treatment, with a success depending on the etiology of the ulcer. Still, future evaluation of the core outcome set in IK-related trials is mandatory for confirmation.

The results of the comparative study between **accelerated and conventional collagen cross-linking for infectious keratitis** suggest that the accelerated photoactivated chromophore collagen cross-linking procedure is as safe and efficient as

the conventional procedure in infectious keratitis treatment. While having comparable costs, the time required for the accelerated procedure is 3 times shorter thus offering significant advantages for patient comfort.

1.3. New concepts and diagnostic methods in posterior segment eye diseases

1.3.1. Introduction

The posterior segment of the eye is situated posterior to the lens, is larger than the anterior segment, and includes the retina, the choroid, the optic nerve, and the vitreous body.

Posterior Segment Eye Diseases (PSEDs) include a large variety of disorders that can variably impact visual function. Although glaucoma, age-related macular degeneration (AMD), and diabetic retinopathy (DR) are the most common ones they do not constitute all PSEDs (Varela-Fernandez et al., 2020). Millions of people worldwide are suffering from retinal and choroid diseases and this number is expected to expand over time due to increased life expectancy and rising incidence of various diseases (eg. diabetes mellitus).

In terms of diagnosis, fundus photography, fluorescein, indocyanine green angiography, and B-scan ultrasound have represented many years the gold standard of retinal imaging. New wide-angle imaging and the new insights offered by optical coherence tomography (OCT) have revolutionized the way we make the diagnosis, the therapeutic decision, and follow up the patient.

Optical coherence tomography angiography (OCT-A) and adaptive optics provide outstanding information about retinal microcirculation. OCT is a non-invasive diagnostic tool that is using the near-infrared light (low-coherence light) to capture cross-sectional images of the retina on a micrometric resolution. The first usage in ophthalmology was performed by Fujimoto et al in 1993, but the instrumentation quickly evolved and rapidly transitioned from the research lab to the clinic. Moreover, due to the continuous improvements in realizing faster, non-invasive ultrahigh-resolution axial measurements of various tissues OCT became the primary standard of care for diagnosing and following retinal eye diseases. Most of the commercial ophthalmic OCT systems used today are spectral-domain (SD-OCT) or swept-source systems (SS-OCT), providing faster scanning, fewer artifacts, and a volumetric image of the retina over a field of at least $6\ \text{mm} \times 6\ \text{mm} \times 2\ \text{mm}$ (Heikka et al., 2020). The image processing allows valuable clinical information, such as maps of the retinal thickness or individual retinal layers. Due to the significant information provided by the OCT technology many retinal disorders were better understood and managed. In some cases, such data further leaned to a major reconsideration and a new classification.

Retinal nerve fiber layer thickness is also of particular interest, as it is an important parameter monitored in glaucoma patients (Pagliara et al., 2008). To facilitate diagnostic decisions, the thickness data obtained is compared to a set of “reference” normative data from healthy subjects. Modern devices use retinal tracking for correcting eye motion during the examination and also to retrace the same imaging area during follow-up. Although retinal imaging is the largest application, OCT is also used to image the anterior segment of the eye (measures the chamber angle for glaucoma diagnosis and corneal mapping to detect corneal abnormalities). Also, the SS-OCT devices allow more sensitive biometry, so important in the intraocular lens (IOL) selection.

The increasing acquisition speed and image quality, have allowed the detection of the blood cells traveling through retinal capillaries. Thus, flow signals are integrated over

depth and displayed as *"en-face"* images in the so-called OCT angiography (OCT-A), in analogy to fundus fluorescence angiograms. OCT-A is currently used as an adjunct for vascular imaging of the retina, choroid, and optic nerve. The ability to rapidly acquire images in a non-invasive and dye-free way concerning the retinal vasculature integrity at different levels and to detect new blood vessels makes this technique very useful in the evaluation of various posterior segment pathologies such as diabetic retinopathy, retinal vascular occlusions, and glaucoma (Koustenis et al., 2017).

Adaptive Optics (AO) retinal imaging provide optical resolutions of 2 μm or less in the living human eye, sufficient to make measurements at cellular and sub-cellular details (Burns et al., 2019). The technique, continuously developed since 1989, is non-invasive, safe, and well-tolerated by the patients.

The usage of AO in the human retina was initially limited to improving measurements of retinal thickness and the identification and quantification of cone photoreceptors. The study of cone changes in aging, myopia, retinal degenerations, and diabetes provided valuable information. The study of retinal vasculature is of particular interest since retinal and systemic diseases have a significant impact.

Useful biomarkers such as the vascular caliber, the vascular wall thickness, the vascular perfusion, the blood velocity or flow, and the oxygen saturation were implemented to monitor the changes induced by diabetes mellitus and systemic hypertension (Agabiti-Rosei et al., 2017). AO can also provide an outstanding visualization of the retinal fiber layer, thus allowing a much earlier glaucomatous damage, and also of the retinal pigment epithelium, mural, glial and other retinal cells.

Deep learning is used in ophthalmology for data analysis, segmentation, automated diagnosis, and possible outcome predictions (Schmidt-Erfurth et al., 2018). The association of deep learning and optical coherence tomography (OCT) technologies has proven reliable for the detection of retinal diseases and improving the diagnostic performance of the eye's posterior segment diseases (De Fauw et al., 2018).

The increase in the number of retinal disease cases has produced an ever-growing demand for retinal image readers. The development of artificial intelligence (AI) and deep learning analysis of retinal imaging can reduce the number of ophthalmologists needed for image interpretation and the time allocated for this procedure. At the same time, AI may increase the efficiency of healthcare providers by establishing the correct and rapid diagnosis of retinal diseases.

This direction of research is reflected in the following published articles:

Moraru AD, Costin D, Moraru RL, Costuleanu M and **Brănișteanu DC**: Current diagnosis and management strategies in pachychoroid spectrum of diseases (Review). Exp Ther Med 20: 3528-3535, 2020 **IF 1.785**

https://doi.org/10.3892/etm.2020.9094

Cristescu IE, Zagrean L, Balta F, **Brănișteanu DC.** Retinal microcirculation investigation in type i and ii diabetic patients without retinopathy using an adaptive optics retinal camera, Acta Endo (Buc) 2019 15(4): 417-422. **IF 0.55**

doi: 10.4183/aeb.2019.417

Moraru AD, Costin D, Moraru RL, **Brănișteanu DC**. Artificial intelligence and deep learning in ophthalmology - present and future (Review), Experimental and Therapeutic Medicine, 2020, 20(4): 3469-3473. **IF 1.785**

https://doi.org/10.3892/etm.2020.9118

Brănișteanu D, Bilha A. Acute posterior multifocal placoid pigment epitheliopathy following influenza vaccination, Romanian Journal of Ophthalmology, 2015, 59(1): 52-58 (Pubmed)

Brănișteanu D, Moraru A. Macular serpiginous choroiditis complicated by macular hole, Oftalmologia, 2014, 58(4): 19-25 (Pubmed)

1.3.2. Aim

On the subjects of **pachychoroid diseases** and **artificial intelligence and deep learning in ophthalmology,** the studies aimed to realize an updated review of the latest achievements and future tendencies, according to the most relevant, reliable, and recent literature data.

In the case of **adaptive optics,** the clinical study aimed to evaluate the retinal microcirculation changes in type I and type II diabetic patients without retinopathy using adaptive optics ophthalmoscopy (AO) and optical coherence ophthalmoscopy angiography (OCT-A).

Last but not least, two uncommon cases of **posterior multifocal placoid pigment epitheliopathy (APMPPE) and serpiginous choroiditis,** are reported with the aim of demonstrating the major implication of modern investigative methods and therapies. In the first case, a possible relationship between various immunizations and APMPPE onset is suspected. The second case reports the unusual rapid development of a macular hole soon after the onset of characteristic clinical features of a rare variant of serpiginous choroiditis.

1.3.3. Material and methods

T**he pachychoroid spectrum of diseases** has been clarified recently after it was first taken into account in 2013 when pachychoroid pigment epitheliopathy (PPE) was described (Warrow et al., 2013). The group of diseases included under this new classification is characterized by retinal pigment epithelial abnormalities, accompanied by choroidal thickening. Recent major advances in the comprehension of the pathogenic process, clinical characteristics, and therapy were achieved mainly due to new imaging techniques.

There is a tremendous interest in the possibility of implementing recent advances in **artificial intelligence and deep learning** in establishing the diagnosis of retinal disorders. AI technologies entered the field of ophthalmology recently and have been in continuous expansion ever since. Soon AI will have an important impact on aiding research to achieve discoveries and improve clinical practice. The benefits and limitations of AI in the field of retinal disease medical management are reviewed.

For the reviews on these 2 hot topics, the authors performed an extensive literature search in the Medline electronic database, using the PubMed interface. The search

process comprised mainly articles written in English, published in the last 5 years, or more if the resulted data was limited. Different keyword combinations, adding to the pathology name words like "diagnosis", "etiology", "treatment" or "classification" were used to refine the search. The title and abstract were subsequently evaluated and the most relevant studies or previous reviews were retained.

In order to investigate the **retinal microcirculation changes in type I and II diabetic patients without retinopathy using adaptive optics ophthalmoscopy (AO) and optical coherence ophthalmoscopy angiography (OCT-A),** an analytical observational study was designed. The study included patients with type I or II diabetes and no diabetic retinopathy (study group) and healthy subjects (control group).

All subjects were completely investigated with the assessment of the best-corrected visual acuity on ETDRS optotype, the intraocular pressure, and the slit lamp eye exam (of both anterior and posterior segment). Subjects whose pupils diameters were less than 4.5 mm received Phenylephrine 10% and Tropicamide 1% to obtain the pharmacological dilation of the pupils.

The rtx1TM AO flood illumination retinal camera (Imagine Eyes, Orsay, France) was used to obtain images of the superior temporal retinal arteriolar branches, close to the optic disc (Figure 1.1). During the acquisition, the subjects were instructed to track the yellow cross of the instrument whose position was decided by the investigator. The comprehensive retinal imaging included also SS-OCT (DRI OCT Triton, Topcon), OCTA (SS-OCT Angio, Topcon), color fundus, and red-free photography (DRI OCT Triton, Topcon). Axial length measurements were obtained with optical biometry (Aladdin, Topcon).

For further analysis, we have included the parameters obtained from one eye of each subject. The analysis of the retinal vessels was acquired automatically from the software offered by the manufacturer (AO detect artery, Imagine Eyes, France) for each analyzed region of interest, selected by the investigator. The measured parameters were the vessel diameter (VD), the lumen diameter (LD), the mean wall thickness (WT), the wall to lumen ratio (WLR), and the cross-sectional area of the vascular wall (WCSA). Vessel diameter is expressed as the algebraic sum of the wall of the arterioles and the vessel lumen. WLR is the ratio between the wall thickness and the lumen diameter, whereas the WCSA is determined based on the lumen diameter and vessel diameter values. Vascular density is defined as the percentage of a given area represented by vessels (Coscas et al., 2016). The vascular densities along the superficial retinal plexus in the parafoveal area were obtained by calculating the mean of the values provided by the proprietary software in a 3x3mm angiocube. The deeper plexuses vessel densities were not included in the analysis as the current software of the SS-OCTA does not provide them. For all variables, descriptive statistics were achieved.

Shapiro-Wilk's test assessed the normality of the studied variables ($p>0.05$) in all three groups. Vessels parameters were compared between groups using one-way ANOVA with Tukey posthoc analysis. When variances were unequal, one-way Welch ANOVA with Games-Howell posthoc analysis was conducted. For non-linear data or not normally distributed, non-parametric tests were applied (Kruskall-Wallis, with standard posthoc analysis). The results are depicted as mean ± standard deviation unless otherwise mentioned. P-values <0.05 were considered statistically significant. IBM SPSS Statistics software (version 23; Armonk, NY: IBM Corp) was used.

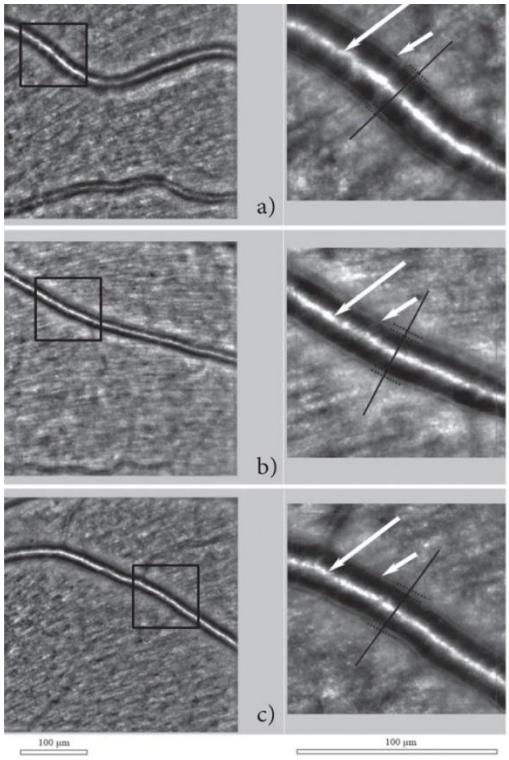


Figure 1.1 - Image of the retinal artery of a patient from the control group (a), from the type I diabetes mellitus group (b) and from the type II diabetes mellitus group (c), with visualization of the walls (short arrows) and lumen (long arrows), employing AO detect artery software.

1.3.4. Results

Concerning the **pachychoroid spectrum of diseases**, the literature research reveals that the etiology remains controversial. It is assumed that autosomal dominant heredity is implicated, together with endogenous and exogenous factors that trigger the onset of the clinical manifestations. The pachychoroid spectrum includes polypoidal choroidal vasculopathy/aneurysmal type 1 neovascularization, pachychoroid neovasculopathy (PNV), PPE, focal choroidal excavation (FCE), peripapillary pachychoroid syndrome (PPS) and central serous chorioretinopathy (CSC). All these disorders have one common pathogenic process that enables their subsequent evolution. The choroid is best studied by enhanced depth imaging-optical coherence tomography (EDI-OCT) and swept source-optical coherence tomography (SS-OCT), both techniques being able to display the deep layers of this tissue and correlate its structural and functional analysis. Optical coherence angiography (OCTA) is a new, non-invasive, 3D imaging technique, which can reconstruct the blood flow in all the vascular layers of the choroid without using contrast substances. These advances allow a better understanding of the pathological processes implicated in the manifestation of various ophthalmological diseases. Using EDI-OCT or SS-OCT it is possible to quantify the choroidal thickness. Thus, a new group of diseases was described, named generally ‘pachychoroid diseases’ which have a sustained, focal or diffuse increase in choroidal thickness over 300 microns, as a common characteristic. The thick choroid is due to the dilatation of Haller's layer vessels, accompanied by subsequent hyperpermeability. Both the vessels in Satler's layer and the choriocapillaris have reduced thickness.

Choroidal thickness, although frequently encountered on OCT, is not the major diagnostic criterion. Each of the diseases included in the pachychoroid spectrum has specific morphological alterations important for establishing the diagnosis. Furthermore, cases that show increased choroidal thickness on OCT, without any changes in the retina or pigmentary epithelium are considered uncomplicated pachychoroid (Dansingani et al.,

2016). The dilated vessels in the Haller layer were named pachyvessels and can be observed on OCT images as large and hyporeflective lumens (Figure 1.2).

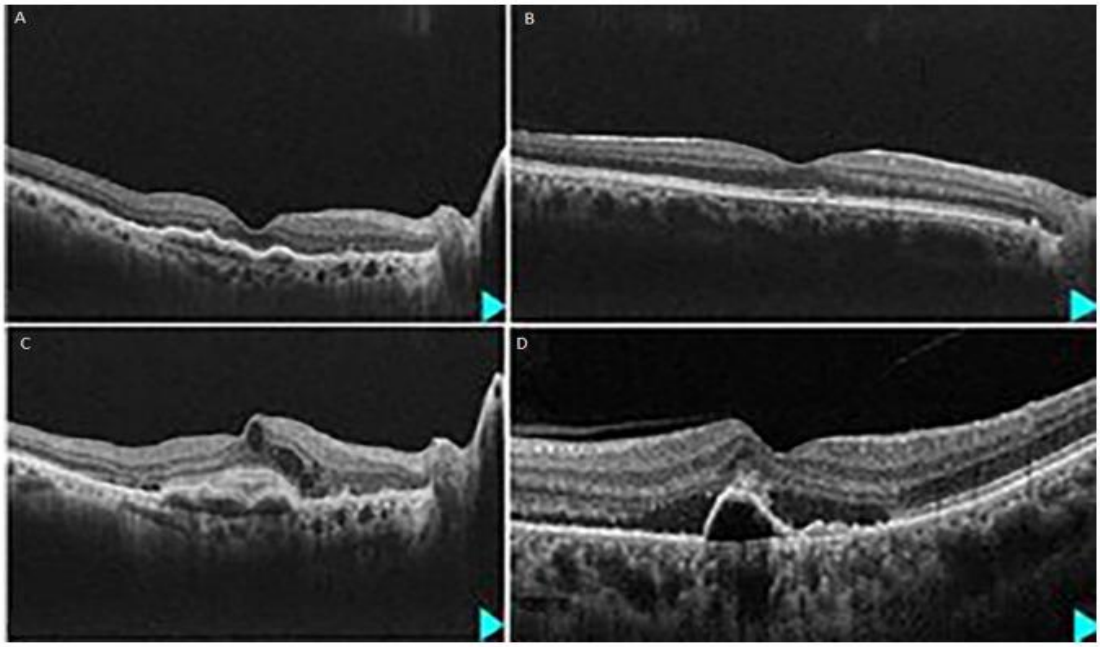


Figure 1.2 - Pachychoroid disease spectrum characteristics:
(A) OCT image of pachyvessels - thickened choroid and dilated choroidal vessels;
(B) OCT image of PPE - thickened choroid and RPE alterations;
(C) OCT image of PNV - irregular RPE detachment over thickened choroid and 'double layer sign' - visibility of both RPE and Bruch's membrane;
(D) OCT image of PCV- thickened choroid, subretinal fluid, and narrow, irregular RPE detachment with polypoid aspect.

An important feature of the pachychoroid disease spectrum is the attenuation of the choriocapillaris and the intermediate caliper vessels in the Satler layer. Due to the thinning of these two vascular layers, some eyes may have a normal choroidal thickness. This attenuation of small and intermediate diameter vessels may signify choroidal ischemia and it is associated, in some cases with retinal outer nuclear layer atrophy, as seen on OCT images and possibly with photoreceptors degeneration. To assess the role of choroidal vasculature features in the development of the disease, automated software that segments the luminal choroidal area from the stromal area on OCT images was developed (Agarwal et al., 2016). Hyperperfusion has an important role in the development of central serous chorioretinopathy (CSC) and pachychoroid pigment epitheliopathy (PPE), by measuring the choroidal blood flow velocity (Saito et al., 2020). Pachychoroid pigment epitheliopathy (PPE) is considered to be a precursor of CSC. In most cases the patients are asymptomatic, presenting changes in the structure of the retinal pigment epithelium, but without the clinical manifestations specific to CSC. Some patients diagnosed with unilateral CSC have alterations specific to PPE in the contralateral eye, which might be proof that PPE represents the first stage of CSC. Some studies report that PPE may evolve into another entity from the pachychoroid spectrum as choroidal neovascular membranes appear, with or without aneurysmal lesions (Pang et al., 2015). A recent study demonstrated that there is a direct relationship between choroidal hyperpermeability, reduced choriocapillaris flow density, and increased choroidal thickness in PPE patients (Sakaruda et al., 2020).

CSC is considered the second stage of the pachychoroid disease spectrum and choroid changes are the main starting point for the physiopathological process. Due to recent advances in technology, it is possible to observe the dilated vessels in the choroid and compare the pathological findings with the asymptomatic fellow eye (Yang et al., 2013). A recent analysis of the choroidal features specific to pachychoroid spectrum diseases revealed that the choriocapillaris vessel density is much lower in CSC compared with uncomplicated pachychoroid and PPE (Demirel et al., 2019).

Pachychoroid neovasculopathy (PNV) may be considered a late complication of the pigment epitheliopathy and CSC. This entity of the pachychoroid spectrum is characterized by the development of type 1 neovascular membrane, located under the RPE and overlying areas of the thick choroid and dilated choroidal vessels. On OCT it is possible to observe an irregular detachment of the RPE, with an almost flat or shallow profile and an inhomogeneous aspect beneath it, which is relevant for the neovascularization located above an area of pachychoroid (Figure 1.2). A 'double layer sign' may be present, representing the visibility of both RPE and Bruch's membrane. OCT angiography shows a network of vessels, typical for an occult neovascular membrane, between the RPE and Bruch membrane (Bousquet et al., 2018).

Polypoidal choroidal vasculopathy, also known as aneurysmal type I neovascularization, was initially described as an area of choroidal capillaries proliferation under the RPE, which develops aneurysms, similar to polyps, at its tip (Imamura et al., 2010). The proliferation then evolves to serous or hemorrhagic detachment of the RPE. This form of the disease, together with the PNV, represents the most advanced stages of the pachychoroid disease spectrum. It is considered that 1 in 10 patients with neovascular AMD is falsely diagnosed and it represents a pachychoroid patient (Freund et al., 2010). A recent analysis of the characteristics of the disease revealed that the aneurysms and the neovascular network are located between the inner Bruch membrane and the RPE and have the potential to bleed in this tight space, thus the 'polyp' designation is not accurate (Dansingani et al., 2018). In the polypoidal choroidal vasculopathy cases, EDI-OCT and SS-OCT show a thickened choroid, choroidal hyperpermeability, subretinal fluid, and narrow, irregular RPE detachments with polypoid aspect (Figure 1.2). OCT-A can show the network of filamentous neovessels occupying the outer retina to the choriocapillaris layer (ORCC) and can successfully replace indocyanine green angiography in detecting the polypoid lesions (Fujita et al., 2020).

Peripapillary pachychoroid syndrome was recently described as an entity of the pachychoroid disease spectrum in which the thickened choroid is located in the proximity of the optic disc (Phasukkijwatana et al., 2018) and, as a consequence, the subretinal and intraretinal fluid is distributed in the nasal macular and peripapillary area.

Focal choroidal excavation may be encountered as a single manifestation of the pachychoroid disease in an eye, or it may be associated with one of the other entities included in this phenotype. There are reports of the association of the FCE with CSC, PNV, and polypoidal choroidal vasculopathy, either in the presenting eye or in the contralateral eye (Lim et al., 2016). Most of these patients are asymptomatic or only have minor visual symptoms, blurring and metamorphopsias, and present myopic refraction. The disease manifests in patients younger than those diagnosed with AMD and without a history of scleral staphyloma.

Referring to the analytical observational study investigating the **retinal microcirculation changes in type I and II diabetic patients without retinopathy using adaptive optics ophthalmoscopy (AOO) and optical coherence ophthalmoscopy angiography (OCTA)** fifty-five participants were included (Table 1.6). The study groups

were divided into type I and type II diabetic patients and healthy controls. The type I DM group included 16 participants (6 females and 10 males), the type II DM, 19 participants (8 females and 11 males), whereas the control group included 20 participants (11 females and 9 males).

Tabel 1.6 - Characteristics of groups included in the study (mean ± standard deviation 95%CI).

	DM I group	DM II group	Control group
N	16	19	20
Sex (female/ male)	6/ 10	8/11	11/ 9
OD/ OS	7/ 9	12/7	11/ 9
Age (years)	38.06 ± 6.87	54.42 ± 9.47	39.60 ± 5.64
Axial length (mm)	23.87 ± 0.81	23.34 ± 0.52	24.16 ± 0.82
Duration of DM (years)	17.38 ± 6.94	7.47 ± 4.27	-

The best-corrected visual acuity was 20/20 or better among all three study groups. The duration of diabetes in the first group (17.38 ± 6.94 years) was significantly longer than in the second group (7.47 ± 4.27 years), $p<0.001$.

In the type II DM group, all subjects required oral hypoglycaemic agents, with one exception, in which diet and exercise were enough to lower the blood sugar level. The subjects met the eligibility criteria, namely, adult age (>18 years old, Caucasians), confirmed diagnosis of type I and type II, respectively, in compliance with the American Diabetes Association, with no lesions of DR (after the ETDRS guidelines), 20/20 or better best-corrected visual acuity (BCVA). Subjects with a positive diagnosis of any ophthalmological disorder (including any eye intraocular surgery, intravitreal injections, laser treatment, macular edema, media opacities, and refractive error greater than 3 spherical dioptres or 2.5 cylindrical dioptres) or other systemic pathology were excluded. The control group included healthy subjects without any past ophthalmological or systemic medical history. All cases were investigated once, in a cross-sectional design.

The values of VD did not vary between the groups ($F(2, 53)=1.714$, $p=0.19$) and neither did those of LD ($F(2, 53)=-0.468$, $p=0.629$), walls ($F(2, 53)=1.057$, $p=0.355$) or WCSA ($F(2, 53)=0.251$, $p=0.779$), as indicated in Tables 1.7 and 1.8.

Table 1.7 - Mean ± standard deviation of the retinal arterioles parameters measured in the diabetic and control groups.

Group	VD (µm)	LD (µm)	Mean wall thickness (µm)	WLR	WCSA
Control	94.87 ± 18.73	76.27 ± 15.36	9.29 ± 2.10	0.24 ± 0.035	2585.1 ± 1057.46
DM I	83.12 ± 22.5	71.31 ± 22.44	9.97 ± 2.19	0.31 ± 0.75	2501.06 ± 1178.34
DM II	92.49 ± 18.18	71.82 ± 14.50	10.33 ± 2.52	0.29 ± 0.58	2758.25 ± 1138.26

Legend: VD=vessel diameter, LD=lumen diameter, WLR=wall to lumen ratio, WCSA=cross sectional area of the vascular wall, DM I= type I diabetes mellitus, DM II= type II diabetes mellitus.

Table 1.8 - Results of the posthoc analysis between the groups included in the study.

Groups	VD (Tukey)	LD (Tukey)	Mean wall thickness (Tukey)	WLR (Games-Howell)	WCSA (Tukey)
Control *vs.* DM I (p value)	0.19	0.67	0.66	**0.01**	0.97
Control *vs.* DM II (p value)	0.92	0.70	0.33	**0.01**	0.88
DM I *vs.* DM II (p value)	0.34	0.99	0.88	0.69	0.77

Legend: VD=vessel diameter, LD=lumen diameter, WLR=wall to lumen ratio, WCSA=cross sectional area of the vascular wall, DM I= type I diabetes mellitus, DM II= type II diabetes mellitus

For the mean wall to lumen ratio (WLR) a one-way Welch ANOVA was conducted and assessed the statistically significant variation of this parameter between groups (Figure 1.3) ($F(2, 53)=6.685$, $p=0.003$).

Post-hoc Games-Howell analysis revealed that WLR was significantly less in the control group (0.24 ± 0.035) when compared to the diabetic groups (for the DM I group mean WLR=0.31 ± 0.75, mean difference=-0.067, 95% CI= -0.113, -0.020; $p=0.01$ and for the DM II group mean WLR=0.29 ± 0.58, mean difference= -0.047, 95% CI=-0.091,-0.004; $p=0.01$, respectively). Interestingly, between the diabetic groups no statistically significant difference was found for the WLR ($p=0.69$).

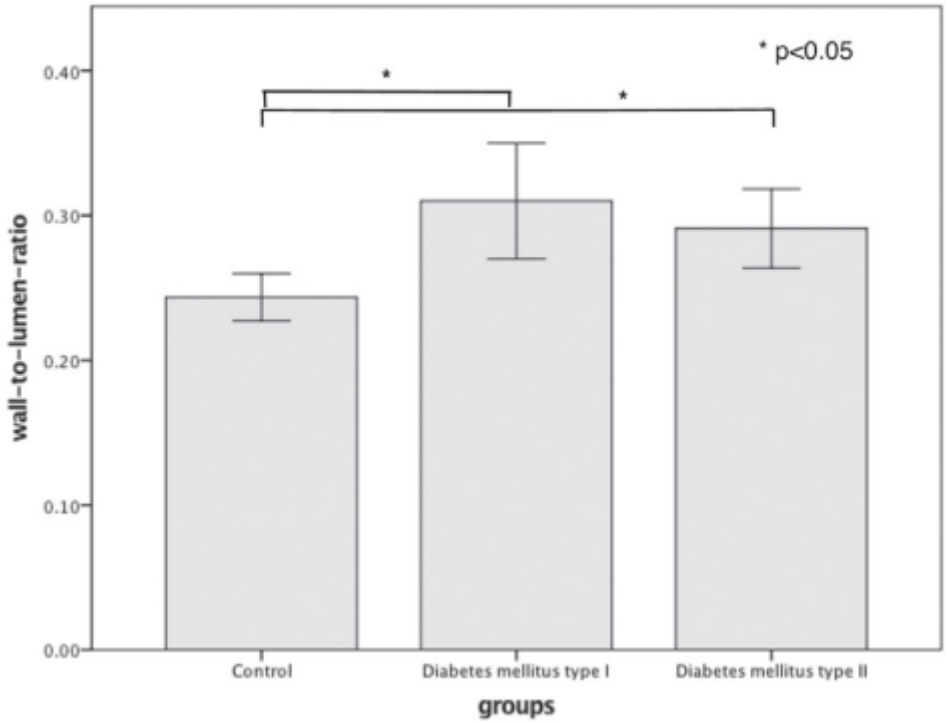


Figure 1.3 - Bar-graph of the wall-to-lumen-ratio values in the three groups included in the study. The error bars represent the standard error of the mean.

The differences in the vascular densities of the superficial capillary plexus were analyzed with a Kruskal-Wallis H test. Distributions of the vascular density values were not similar in the three groups included in the study, as assessed by the visual inspection of the box plots of the data. Significant variations between the three groups ($H(2)=13.236$, $p=0.001$) (Figure 1.4) were found.

Pairwise comparisons were assessed using Dunn's procedure with a Bonferroni correction for multiple comparisons. Values are mean ranks unless otherwise stated. This posthoc analysis revealed statistically significant differences in vascular density values between controls (37.37) and type I diabetic patients (17.63) ($p=0.001$), but not between the controls and the type II diabetic patients (27.40) ($p=0.156$) and between the two groups of diabetics ($p=0.207$).

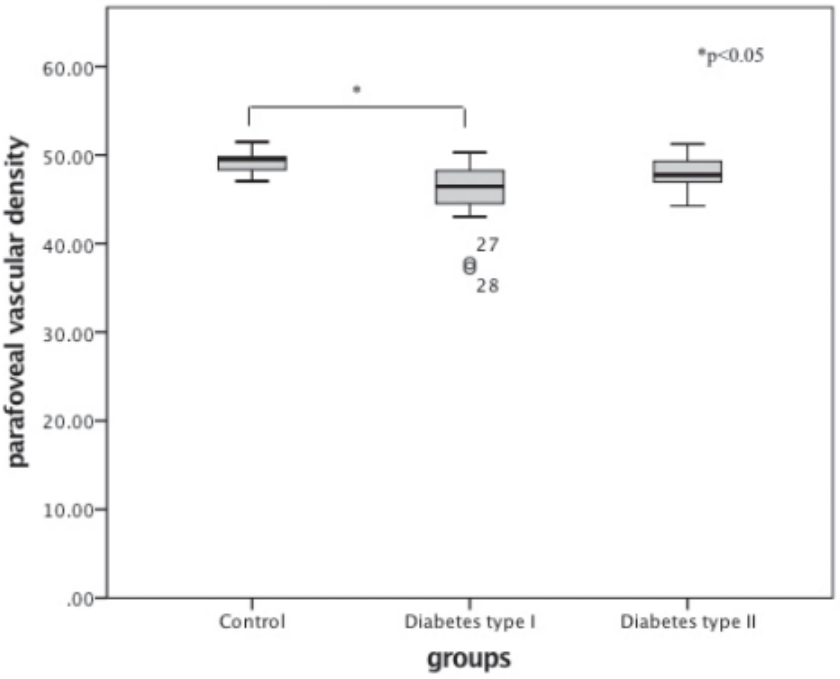


Figure 1.4 - Box-plot of the values of vascular density, with significant outliers plotted on the graph as points outside the box plots.

Concerning the review on **artificial intelligence and deep learning in ophthalmology**, investigative efforts have been identified for utilizing **deep learning algorithms** as tools for automated reading of OCT images for diagnostic purposes. Numerous algorithms have been developed and various landmarks identified to recognize non-pathologic OCT images so automated OCT reading has shown promising results in DME and identifying exudative AMD (Lee et al., 2017, Kermany et al., 2018, Liu et al., 2011). In a recent evaluation, 97.8% sensitivity, 97.4% specificity, and 0.99 AUROC (area under the receiver operating characteristic) curve for detecting referable AMD were noticed (Kermany et al., 2018). An AUROC curve score over 0.7 relates to outstanding results, while under 0.5 proves no discrimination was made. Thus, DL can accurately diagnose AMD in the early stages. Automated OCT reading has also been proven useful in the diagnosis of diabetic retinopathy, central serous chorioretinopathy, polypoidal choroidal vasculopathy, and macular holes (Syed et al., 2016, Xu et al., 2017). Physicians may benefit from OCT reading algorithms as a triaging mechanism and a guide in the therapy decision-making process.

Deep learning algorithms have been created to minimize the clinical reasoning variability and inconsistency in diagnosing various retinal diseases. In retinopathy of prematurity (ROP), many of them show accuracy in detecting ‘plus’ or ‘pre-plus’ disease (lower degree of vascular tortuosity). Recently, Brown et al. used deep learning for training a deep convolutional neural network on a set of 5,511 images. These images were previously rated by experts as ‘plus’, ‘pre-plus’, or ‘normal’. The validation of a set of 100 retinal images showed: 93% sensitivity and 94% specificity for the detection of ‘plus’ disease and 100% sensitivity and 94% species in detecting ‘pre-plus’ disease. Similar studies showed that deep learning may be able to minimize inter-observer variability. In diabetic retinopathy recent literature data showed that machine learning classes (MLC, support vector machine, multiple layer perceptron classes, and radial basis function neural network) can recognize various stages from images, with results comparable to those

obtained when the retinal images were analyzed by ophthalmologists. The software can recognize hemorrhages, exudation, microaneurysms, cotton-wool spots, and neovascularization, build a model and based on it can classify DR in stages. In AMD the screening system is capable of differentiating between normal and AMD in OCT images. They obtained a peak sensitivity of 0.926, a specificity of 0.937, and an AUROC of 0.9746 (Lee et al., 2017). In a larger study, Treder et al. used 1112 OCT images. They created MLC software that differentiates a healthy macula from one showing exudative AMD and obtained a sensitivity of 1.00 and a specificity of 0.92. Some studies are focused on AMD grading and predicting the final visual acuity by using OCT images. The presumptive prognosis may influence the clinician's decision in establishing the treatment of the disease. Schmidt-Erfurth et al. predicted best-corrected visual acuity at 1 year immediately after establishing the diagnosis of the disease with the help of AI. The registered error was 12.9 letters. They trained their MLC on data sets from 614 eyes (2456 OCT scans). Aslam et al., in a similar study, reported a mean error of 8.21 letters. They analyzed only 847 OCT scans. Burlina et al. made imaging assessment more efficient through grading systems, which have the potential to function as a decision support system for clinicians. The study group developed software for different MLCs using more than 130,000 OCT images from 4,613 patients. They concluded that deep CNN is the most precise. They obtained an accuracy of the analysis comprised between 0.884 and 0.916.

Some studies looked at the role of AI as support to therapy decision-making in AMD (if and when the anti-vascular endothelial growth factor, anti-VEGF, treatment is necessary). AI and predictive treatment technology have been proven as a useful addition to clinical practice. This is possible by training MLCs using OCT imaging. Prahs et al., Chakravarthy et al., and Schlegl et al. found that their deep learning CNN was able to correctly predict the need for anti-VEGF therapy (intravitreal injection) in 95% of the cases, similar to an average specialist. The researchers analyzed different features of the scan, particularly retinal fluid presence. Central retinal thickness and fluid localization are important biomarkers in OCT images. Schmidt-Erfurth and Waldstein performed an analysis able to predict the functional prognosis (best-corrected visual acuity outcomes) in neovascular AMD patients about to receive ranibizumab treatment. Bogunovic et al. applied an algorithm to 61 eyes with AMD. This algorithm used OCT biomarkers to reasonably predict drusen regression over the next 2 years. They obtained an AUROC curve of 0.75. Drusen regression has been shown to precede the progression of non-exudative AMD.

Regarding the 2 clinical cases with an unusual presentation, their evolution is presented below.

An 18-year-old female patient was referred for a second opinion and diagnosed with **acute posterior multifocal placoid pigment epitheliopathy (APMPPE)** due to a painless significant bilateral decrease of vision, with a short delay between the eyes, for one week. Other symptoms included moderate photophobia, metamorphopsia, and intermittent headaches. The medical history was unremarkable except for sporadic episodes of drug and food allergies. The patient also described moderate flu-like prodrome, two weeks before, soon after having a seasonal anti-flu immunization. Physical examination was within normal limits and the patient no longer had fever or malaise. Best-corrected visual acuity (BCVA) was 0.2 in the right eye (RE) and 0.16 in the left eye (LE). Intraocular pressure was normal and no refraction error was determined on refractometry. The pupillary reflexes were normal in both eyes. The color vision evaluation pointed toward a discrete red-green deficiency. External and slit-lamp

examination of the anterior segment of both eyes was unremarkable, except for a slight ciliary injection of the conjunctiva. The fundus examination revealed a clear vitreous, not detached posteriorly, a normal optic disc, and normal retinal vessels. There were numerous yellow-white placoid lesions in the macula and the mid periphery, slightly prominent and circumscribed, located in the deep layers of the retina, better observed with a red-free filter (Figure 1.5). The retinal periphery was normal.

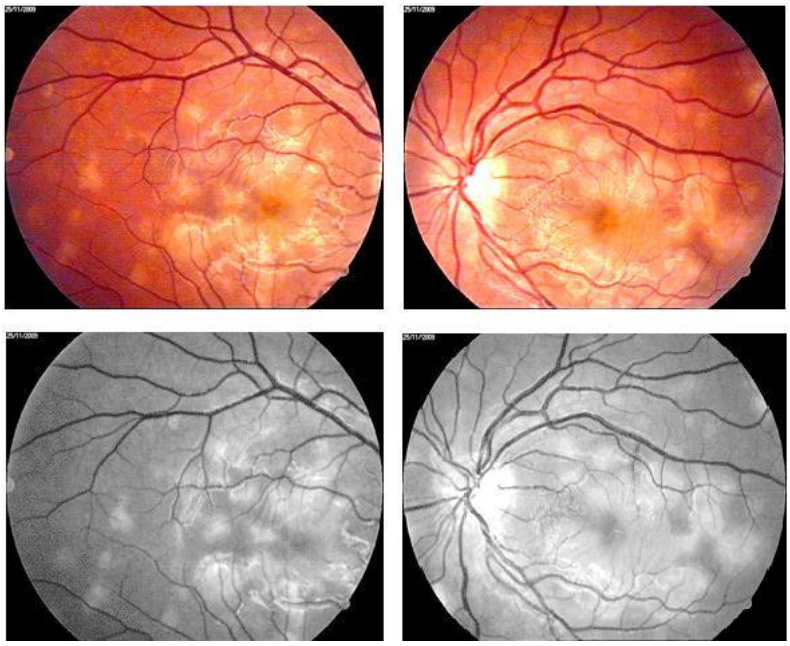


Figure 1.5 - Color and red-free fundus photography OU during the first visit.

The patient did not consent to the fluorescein angiography evaluation because of her multiple foods and drug allergies. Multiple paracentral scotomas were revealed on visual field evaluation. Spectral-domain optical coherence tomography (B-scan and topography) confirmed the discrete retinal thickening at the site of the lesions with dome-shaped elevations of the ellipsoid zone band, hyper-reflective alterations at the level of the photoreceptor layer, and RPE. The inner retinal layers were normal. No subretinal fluid was detected as shown in Figure I.6.

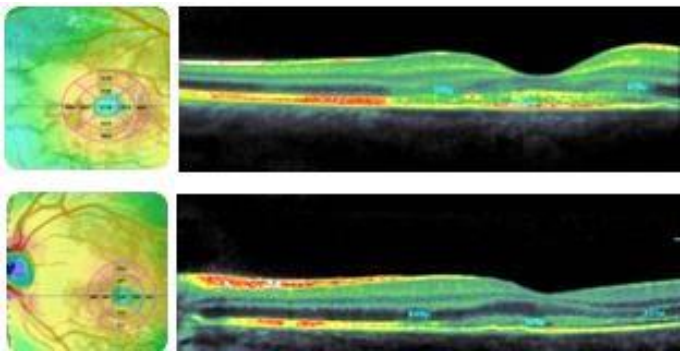


Figure 1.6 - SD-OCT in OU during the first visit: macular map and B-scan.

Ancillary testing included laboratory and imagistic evaluation, compulsory for the diagnosis of comorbidities and differential diagnosis. The complete blood count, the

erythrocyte sedimentation rate, rheumatoid factor, antinuclear antibodies, anti-toxoplasma antibodies, angiotensin-converting enzyme, purified protein derivative tuberculin skin test, immunity assessment, anti-cytomegalovirus antibodies, anticardiolipin antibodies, Lyme disease antibodies, and chest X-ray were all in normal limits. Neurological examination including cerebral computed angiotomography for the intermittent headaches was also unremarkable. Due to significant macular involvement and vision loss, oral prednisone 0.5 mg/kg/day was administered and decreased gradually for over 4 weeks. The condition evolved favorably under treatment with gradual remission of symptomatology and recovery of visual function. Clinical evaluation at 1 year, 3 years, and 5 years after the acute episode confirmed the remission of retinal lesions, the absence of recurrences, and the restoration of the visual field, color vision, and visual acuity (BCVA RE=1, BCVA LE=0.9 after 1 year, and BCVA RE=1.3, BCVA LE=1 after 3 and 5 years). On B-scan, the dome-shaped elevations of the ellipsoid zone significantly flattened at 1 year and disappeared at 3 and 5 years follow-up. Despite obvious atrophic changes in the RPE corresponding to the initial lesions, the macular topography showed no abnormality (Figure 1.7). Minimal residual RPE irregularity was persisting (Figures 1.8 and 1.9).

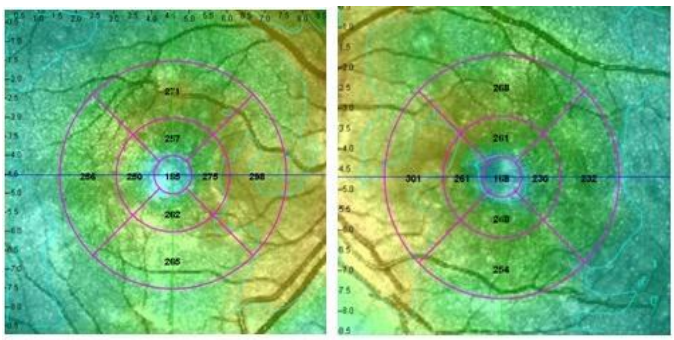


Figure 1.7 - Macular map OU after 5 years of follow-up.

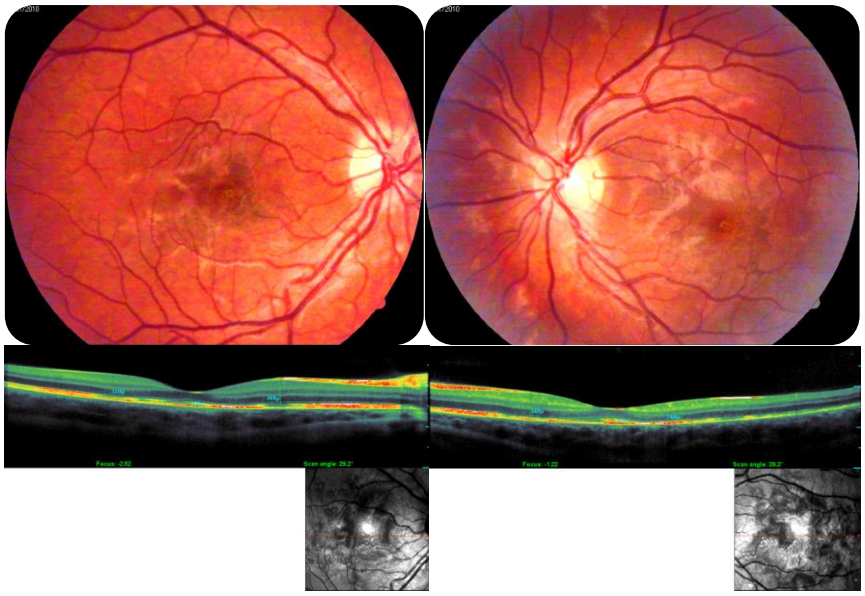


Figure 1.8 - Fundus photography and macular B-scan OU after 1 year of follow-up.

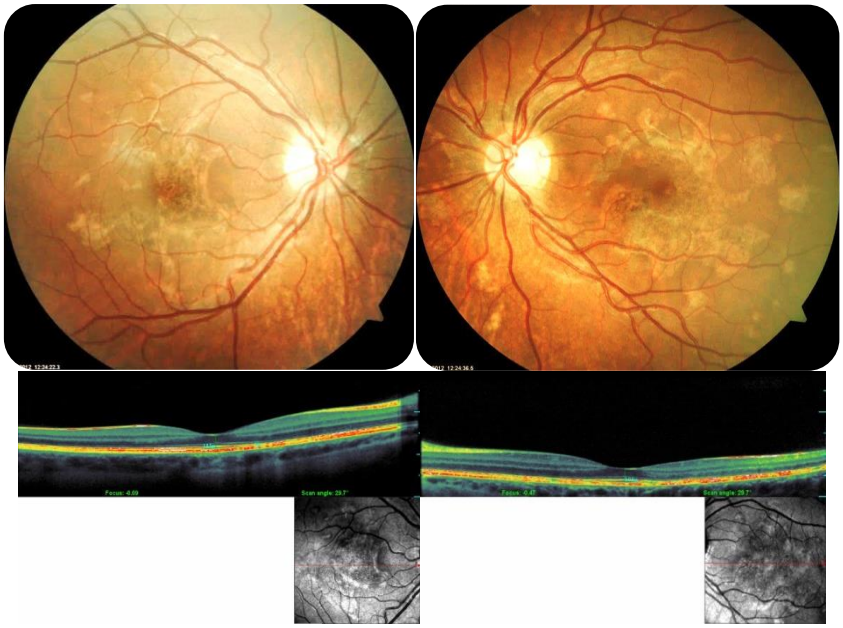


Figure 1.9 - Fundus photography and macular B-scan OU after 3 years of follow-up.

1.3.5. Discussions

Concerning the **pachychoroid spectrum of diseases**, the literature agrees that the main pathogenic mechanism leading to all the abnormalities associated with clinical manifestation is choroidal dysfunction. The increased choriocapillaris permeability generates, in time, the attenuation of this structure with pigmentary changes in the RPE and VEGF expression. As a result, the disease progresses to neovascular membranes and exudation (Dansingani et al., 2018). The occurrence of the neovascular membranes and their evolution is different from those associated with AMD, as choriocapillaris chronic inflammation and attenuation are the triggers of the disease (Kitaya et al., 2003). Although the pathogenesis of pachychoroid disease remains controversial, there is some evidence that an altered steroid metabolism is involved. It is known that the choroid expresses receptors for mineralocorticoid and the continuous stimulation of these receptors leads to an increase in its thickness (Zhao et al., 2012). Also, prolonged administration of corticosteroids may have as a result occurrence of PPE features, followed by CSC development, confirming the supposition that dexamethasone intravitreal implants alter choroidal functionality (Ersoz et al., 2018). With the increased hydrostatic pressure at the level of the Bruch membrane, the RPE complex seems to be the most important factor in the development of the pigment epithelium and neurosensory retinal detachments. As a result of the choriocapillaris attenuation and the subsequent ischemia, there is an overexpression of pro-angiogenic factors that stimulates the development of a choroidal neovascular membrane. At the same time, the increase in the volume of the Haller's layer vessels and the mechanical pressure on the RPE causes atrophy, pigmentary changes and ruptures of the Bruch membrane, facilitating the occurrence of CNV. A genetic phenotype may be common to all the entities included in the pachychoroid spectrum and although different entities of the pachychoroid spectrum appear to be stages of the same disease, the evolution from one stage to another is not mandatory.

In terms of the treatment, photodynamic therapy using verteporfin (PDT) may be used for chronic CSC, but some adverse effects have been reported, such as CNV

formation and alterations of the electroretinography aspect. Half-dose PDT is safer and more effective in reducing choroidal thickness, especially at the level of the Haller layer (Izumi et al., 2017). The use of anti-VEGF agents is controversial in CSC. Choroidal ischemia accompanies CSC, with a subsequent rise in the level of VEGF, thus the administration of anti-VEGF agents seems to be beneficial. Although in some reports (Chhablani et al., 2015) most cases of FCE, PNV, and polypoid choroidal vasculopathy evolved favorably after the administration of intravitreal anti-VEGF agents, the effect of anti-VEGF agents on persistent subretinal fluid and macular edema secondary to CSC was not demonstrated in large trials (Staurenghi et al., 2018, Chung et al., 2013). Combined therapy (PDT and anti-VEGF agents) is more efficient in controlling the neovascular membranes than monotherapy. This effect is due to both the diminishing of exudation by the intravitreal anti-VEGF agent and the thrombosis induced by PDT in the neovessels (Yong et al., 2015).

Regarding **adaptive optics ophthalmoscopy,** it is a new imaging tool that allows the visualization of photo-receptors (Cristescu et al., 2019) and vessels in the human living retina, at a histological resolution. In our research, an AO fundus camera was used to appreciate the differences in retinal arterioles parameters in type I and II diabetic patients with no DR and healthy volunteers. Moreover, vascular densities were measured. The results suggest that diabetic patient without retinopathy present signs of early dysfunction of retinal arterioles, as depicted by WLR. Subjects with hypertension or any other vascular disorders were excluded to avoid any interaction between the retinal vessel parameters and these conditions. Interestingly, no statistically significant difference was found between the two diabetic groups concerning the studied parameters. To our knowledge, this is the first study in the literature to compare the retinal arterioles parameters between type I and type II diabetic patients using an AO retinal camera. The results of this study are confirmed by the literature that describes as well arterial remodeling signs in diabetic patients with no retinopathy (Zaleska-Zmijewska et al., 2019). Lombardo et al. studied the lumen of parafoveal retinal capillaries in eyes with nonproliferative DR and healthy controls with an AO retina camera and the vessels were found to be narrower in diabetic subjects than in healthy ones. Similar results were obtained by Zaleska et al., when comparing the arteriolar parameters in type I diabetic patients and controls using the AO. An increased WLR depicts very well the arterial remodeling process in diabetes mellitus that may encompass the thickening of the wall, the narrowing of the lumen, or both. In diabetic patients, retinal arterioles narrow their lumen because of fibrosis and the growth of smooth muscle cells (Trost et al., 2016). These changes represent an early stage of diabetes-associated vascular impairments. Nevertheless, although there was a significant difference in duration of diabetes and subjects' age between the two diabetic groups included in this study, the retinal arterioles parameters assessed by the AO retinal camera did not differ much between the two groups.

Furthermore, another result of this study proved a lower vascular density in the superficial capillary plexus in the parafoveal area in type I diabetic patients compared to controls, using OCTA. In diabetes, the lesions in the microcirculation appear before any clinical sign of retinopathy, and OCTA angiography is enabling early detection of these retinal vascular changes (De Carlo et al., 2015). Vascular density is a biomarker that allows the quantitative assessment of vascular disorders. In diabetic retinopathy, vascular density decreases in both superficial and deep plexuses (Khadamy et al., 2018). Yet, some studies showed a lower vascular density in the deep capillary plexus only (but not superficial capillary plexus or choriocapillaris) in diabetic subjects without DR (Kim et

al., 2018, Vujosevic et al., 2019). The significantly lower vessel density found in the type I diabetic group, but not in the type II diabetic group when compared to controls, might be connected to the longer duration of the disease in the first group. Lacking OCT-A data from the deep capillary layers, no conclusion can be made concerning the extent of microvascular lesions induced by diabetes in the two study groups. However, the vascular density and the foveal avascular zone are the most common OCT-A parameters used for the early detection of DR. Its severity, the visual function, and the response to treatment are correlated to these biomarkers (de Barros et al., 2017, Mastropasqua et al., 2017).

Adaptive optics ophthalmoscopy proved to be a valuable instrument to quantify the retinal microvascular changes associated with diabetes mellitus in patients with no DR (Gallo et al., 2018). Thus, prognostic information might be obtained concerning the incidence and progression of DR. In this study, the AO retinal camera was able to reveal retinal microvascular lesions in the type II diabetic patients group, whereas OCTA found no differences when compared to the control group.

The small sample sizes might represent a source of bias. Besides larger study groups, future studies should analyze the superficial, deep capillary, and choriocapillaris layers with OCT-A, including correlations of the vessels data with the blood parameters, and the diabetes duration.

As regards **artificial intelligence**, more and more studies demonstrate that **deep learning** can be utilized to extract data that ophthalmologists cannot read. Deep learning results in image analysis comparable to that of human performance. Although many studies have validated the potential of neural networks in diagnosing various retinal diseases, AI usage in clinical practice might present some potential risks. Some software programs are based on algorithms that lead to high false-negative rates of detection of retinal diseases. Improper interpretation of the false-positive results may lead to diagnostic errors and could be clinically disastrous for patients' vision. Sometimes the ophthalmologist cannot evaluate the metrics values used by the AI computer software to analyze the clinical data. The method by which a computer algorithm came to its conclusion, the reasoning process, is not always obvious. It is possible that remote screening (patient's home screening) by automated AI systems, may become a problem due to a lack of patient confidence. Some studies show that many patients do not trust computer-aided diagnosis and prefer in-person ophthalmology visits (Keel et al., 2018). Also, there is a risk for doctors to become addicted to technology and lose diagnostic abilities. For some particular situations, when a physician disagrees with the results obtained by deep learning assessment, or when a patient does not receive counseling related to the required treatment it is necessary to introduce and apply medicolegal and ethical regulations. All these potential shortcomings highlight the need for continuous improvement in AI technology. Shortly, AI will become more involved in the decision-making regarding scientific investigation, diagnosis, and therapeutic management. Tele-ophthalmology applications can transmit information to less developed regions that face a shortage of specialists (Armstrong et al., 2020). Already, a hybrid algorithm, with high sensitivity and specificity, named IDx-DR, approved by FDA as a low to moderate risk device, is used for diabetic retinopathy screening, aiding the management of patients that need a referral to an ophthalmologist. AI can quickly analyze large databases. Based on these analyzes, AI can explore the associations between disease features that may not be easily obvious to humans. The clinical analysis of the ophthalmologist supported by the DL analysis results will improve the individualization of the medical management, for the benefit of the patients. AI also plays an important role in scientific research. With the help of AI, the features of newly discovered eye diseases can be identified (Keel et al., 2018).

It is expected that AI algorithms will help in identifying new biomarkers, specific to each disease, as they can search for characteristics themselves and are not limited to only recognizing clinical features. Ongoing research aims to develop autonomous software able to diagnose simultaneously AMD, diabetic retinopathy and glaucoma, predict progression, and recommend personalized treatment.

Regarding the unusual clinical cases presented, "**white dot syndromes", including APMPPE**, are rarely seen in current clinical practice as the estimated incidence is around 0.45 per 100.000 inhabitants. APMPPE has a higher predilection for Caucasians (80%), mostly between 16 to 40 years of age, without a predilection for one of the sexes. Statistically, APMPPE is more frequently associated with autoimmune diseases, with half of the patients having psoriasis in their medical history (Abu-Yaghi et al., 2011). Recent studies have pointed to a higher association with HLA-B7 and HLA-DR2, suggesting a genetic predisposition to this disease (Baxter et al., 2013). The pathogenesis of APMPPE remains largely unknown. A delayed type hypersensibility associated vasculitis, which affects the choroidal terminal lobules and systemic vasculature, is incriminated (Park et al., 1995). This obstructive vasculitis located at the level of the choroidal terminal lobules can induce irreversible ischemic changes in the outer layers of the macula. This pathogenic theory is supported by the association of APMPPE with other vasculitis (cerebral angiitis, thyroiditis, nephropathies, and erythema nodosum) (Senanayake et al., 2008). The possible relationship between APMPPE and other vasculitis, Lyme disease (Bodine et al., 1992), adenoviral disease, mumps (Birnbaum et al., 2010), sarcoidosis, pulmonary tuberculosis (Abu El-Asrar et al., 2002), or other rheumatologic conditions (Abu-Yaghi et al., 2011), raised the need for specific investigations to elucidate the diagnosis, to identify associated pathological conditions and to lead towards appropriate therapy management. Moreover, Darugar et al. published a case of sarcoidosis with APMPPE as the initial manifestation.

There is documented information in the literature that APMPPE can be triggered by vaccination against B hepatitis (Brezin et al., 1995), meningococcal type C (Yang et al., 2005), human influenza (Mendrinos et al., 2010), swine flu (Hector et al., 1978) or varicella (Fine et al., 2010). In these cases, there is an obvious implication of activated T lymphocytes and type IV hypersensitivity. Recurrences are at least theoretically related to hypersensitivity towards various pathogens. Approximately one-third of the patients have mild or moderate flu-like symptoms that precede with a few days the onset of visual impairment. The fever, malaise, and gastrointestinal symptoms may delay the diagnosis of APMPPE.

APMPPE is mainly diagnosed on clinical findings and evolution, there has been no specific laboratory testing so far. Active lesions show early hypofluorescence followed by late hyperfluorescence on fluorescein angiography. Healed lesions generate a window defect. Indocyanine green angiography has more accuracy than fluorescein angiography in revealing choroidal defects in the early stages corresponding to choroidal lobules nonperfusion. RPE lesions slowly become autofluorescent in the remission phase. The high-resolution OCT aspect in the early stages shows dome-shaped elevations of the ellipsoid zone band. Hyperreflectance above RPE could be explained by ischemic edema or accumulation of inflammatory cells. Subretinal fluid accumulation is uncommon. With remission, the dome-shaped lesions flatten, the outer layers partially recover and the RPE shows residual irregularities (Cheung et al., 2010). Differential diagnosis of APMPPE mainly concerns white dot syndromes, toxoplasmosis, neuroretinitis, syphilitic chorioretinitis, and Vogt-Koyanagi-Harada disease.

Several ocular complications can interfere with the long-term prognosis. The most severe ones are secondary choroidal neovascularization (Bowie et al., 2005), the appearance and persistence of subretinal macular fluid, and central vein occlusion (Allee et al., 1998). Rarely, retinal vasculitis, papillitis, and cystoid macular edema (Yenerel et al., 2008) have also been described. Recurrences have been cited in up to 50% of the cases (Taich et al., 2008). The concomitant central nervous system impairment due to granulomatosis or another vasculitis can lead to neurological disorders with a significant impact on morbidity. The patient may develop headaches, paresthesias, paresis, meningoencephalitis, cavernous sinus thrombosis, or cerebral infarcts. Cases with important cerebral ischemic complications, mainly in men, have already been published (Yunker et al., 2008). El Sanhouri et al. published the case of a patient with APMPPE, Chron's disease, and important headaches in which rapid prednisone taper led to death through multiple cortical infarcts in a short period (El Sanhouri et al., 2012). In such severe cases, high-dose intravenous steroid therapy is indicated, and an immunosuppressive drug may be associated.

APMPPE is generally considered to have a good prognosis and does not require any treatment due to spontaneous remission. Although there is no evidence that systemic corticosteroid therapy influences final visual acuity, corticosteroid administration is recommended when the macula is significantly involved or/ and when systemic comorbidities are associated. Fiore et al. made an analysis of the evolution of visual acuity in APMPPE patients and concluded that visual prognosis is not as good as studies initially reported. In the case of foveal involvement, there is a probability of less than 40% to recover a visual acuity higher than 20/ 25, while eyes without foveal damage have a chance of almost 90% of obtaining a higher visual acuity than 20/ 25 (Fiore et al., 2009).

1.3.6. Conclusions

Since the inclusion of the **pachychoroid spectrum of diseases** in the ophthalmologic nomenclature in 2013, significant progress was achieved in the latest years in understanding the pathogenesis and classification mainly due to newer diagnostic tools. Additional research is needed to better understand the mechanisms that determine the development of a certain manifestation of the disease and the progression from one form to another, to improve the therapeutic management and target the pathogenic mechanism of each clinical form.

The investigation of the retinal vessels with state of art imaging techniques **like AO and OCT-A** might help to better understand the pathophysiological mechanisms of DR (in DM I and DM II), the chronology of the retinal vasculature lesions, and their connection to risk factors and blood parameters. They both give valuable information on the topological and geometrical changes of the retinal vessels in diabetes before any clinical sign of DR. An increased wall to lumen ratio (WLR), as demonstrated with the adaptive optics, significantly indicates the microvascular disease in diabetes mellitus.

Artificial intelligence and deep learning algorithms using both OCT and fundus images have already revolutionized the techniques and methodologies of image analysis. Optimizing these combined technologies will accelerate the progress in this area. Currently, there are software programs that standardize OCT images from various devices and the results of these software packages are comparable when the appropriate metrics are used consistently. Nevertheless, large databases, using real patient data, are required

to optimize the performance of this type of analysis. Thus, computer-assisted screening, diagnosis, and prediction of ocular diseases will reach new frontiers. AI has the potential to increase patient access to both clinical screening and diagnosis and to decrease healthcare costs, especially when the risk of the disease occurrence is high or the communities confront with low financial resources. However, legal regulations and solving of the reproducibility issues are required before AI-based screening is incorporated into clinical practice.

The reported case of **acute posterior multifocal placoid pigment epitheliopathy (APMPPE)** is unusual due to the onset strongly correlated with anti-influenza vaccination. The good prognosis in such rare pathology was confirmed by the favorable evolution under treatment with fully and stable recovery after 5 years of follow-up.

1.4. New therapeutic options in posterior segment eye diseases

1.4.1. Introduction

Uveal melanoma (UM) is a rare condition accounting for only 5% of all primary melanoma cases. Still, it is the most frequently diagnosed primary intraocular malignant tumor in adults. Almost 90% of the tumors involve the choroid and only a small percentage affect the ciliary body or the iris. There is a consistent difference in incidence between different regions with individuals of northern European descent having a significantly higher risk as compared to Hispanics, Asians, and Blacks. Among the many risk factors, mutations in the G protein subunit alpha Q (GNAQ) or G protein subunit alpha 11 (GNA11) genes and different receptors are highly suggestive. While iris melanoma can easily be noticed by the patient itself or diagnosed at a routine slit-lamp evaluation, a consistent percentage of posterior uveal tumors are incidentally diagnosed at funduscopic evaluation as they can evolve silently for years, especially if located in the periphery. Uveal melanoma classifications have been updated and rely mostly on the tumor size (thickness and basal diameter) and also on intraocular and extraocular extension. The differential diagnosis with pseudomelanomas is carried out according to the tumor aspect and position. Iris melanoma has a better prognosis and a lower mortality rate as compared to choroidal melanoma which has a much higher rate of metastasis (50% of the patients) and a subsequently limited life expectancy from 6 to 12 months. While modern conservative therapeutic options for the primary tumor, relying on different surgical excision techniques and/or irradiation therapies, offer good local tumor control, the treatment options for metastatic disease, although numerous, are still deceiving in preventing a fatal outcome.

This direction of research is reflected in the following published articles:

Branisteanu DC, Bogdanici CM, Branisteanu DE, Maranduca MA, Zemba M, Balta F, Branisteanu CI and Moraru AD: Uveal melanoma diagnosis and current treatment options (Review). Exp Ther Med 22: 1428, 2021 **IF 2.447**

https://doi.org/10.3892/etm.2021.10863

Gene therapy (GT) is currently on a continuously progressive trend and over the past two decades, promising advancements have been made in the treatment of inherited and previously intractable diseases. Gene therapy techniques have in common the insertion of a foreign DNA fragment into host cells, aiming to modify the expression of

proteins by the target cells. The eye is an effective target for genetic therapy, as it has a privileged immune status, it is easily accessed for medication delivery and it is affected by several inherited disorders. The retina is in particular considered for gene therapy because it can be visualized with ease, it does not have lymphatic vessels or a direct blood network for the outer layers and its cells do not divide after birth, and thus the transgene expression is not affected. Genetic manipulation techniques include gene inactivation, gene augmenting, and gene editing. Gene inactivation aims to block the production of an abnormal protein that is produced in the target cell and replace it with a therapeutic protein. This technique is mostly used in inherited retinal diseases that are associated with gain-of-function. Gene augmentation is particularly used in diseases characterized by loss of function, thus aiming at replacing a 'missing' protein in the target cell. Gene editing consists of marking DNA in the target cell for replacement. The technique is known as clustered regularly interspaced short palindromic repeats (CRISPR) and uses RNA linked to the Cas9 enzyme, to identify, cut, and remove specific portions of DNA that are to be replaced. This technique has the disadvantage of possibly affecting other portions of DNA, thus creating novel mutations. Over the past two decades, major improvements in surgical techniques necessary for the delivery of modified genes to the retinal tissue have been achieved, thus contributing to the development of novel and revolutionary therapeutic strategies.

This direction of research is reflected in the following published article:

Moraru AD, Costin D, Iorga RE, Munteanu M, Moraru RL and **Branisteanu DC:** Current trends in gene therapy for retinal diseases (Review). Exp Ther Med 23: 26, 2022 **IF 1.785**

https://doi.org/10.3892/etm.2021.10948

Neovascular glaucoma (NVG) is one of the most refractory forms of glaucoma, caused by various ocular and occasionally systemic conditions that produce retinal ischemia. NVG often appears as an end-stage disease, resulting in blindness, continuous pain, and eventually loss of the eye. In this stage, the objective of the treatment is to lower the intraocular pressure (IOP) to relieve the pain and preserve the globe. Numerous treatments have been attempted for lowering IOP in NVG, but no consensus exists to date regarding the most effective and safest procedure. Trabeculectomy with antimetabolites, aqueous shunt implantation, and cyclodestructive procedures are the main methods used to treat high IOP in NVG. For many years, a variety of methods resulting in cyclodestruction have been used to reduce the aqueous formation and, subsequently, the IOP. Non-penetrating and penetrating cyclodiathermy were introduced in the 1930s, cyclocryotherapy in the 1950s, and later high-intensity focused ultrasound, but all of these have been abandoned due to the high risk of devastating complications. Cyclophotocoagulation is a form of cycloablation that focuses high-intensity laser energy at the level of ciliary epithelium, where it is absorbed by melanin and transformed into heat with a coagulative effect, resulting in the reduction of aqueous production and, consequently, in lowering of the IOP. Although numerous types of lasers have been used, diode lasers are currently considered to be the most appropriate. The diode laser emits a beam with a wavelength of 800-850 nm, which is best absorbed by the melanin in the pigmentary epithelium, with less energy affecting the sclera. The energy delivery periods are quite long, 2-3 sec, and therefore, high energy is transferred to the ciliary stroma, with

coagulative effects. Traditionally, transscleral cyclophotocoagulation (TSCPC) continuously delivers laser energy. Continuous-wave transscleral cyclophotocoagulation (CW-TSCPC) is effective in lowering IOP, but has a risk of important complications such as a decrease in visual acuity (VA), hypotony, chronic uveitis, and phthisis bulbi. These complications are likely the result of damage to the surrounding tissues due to the spread of thermal energy. Another technique, micropulse transscleral cyclophotocoagulation (MP-TSCPC), involves using a novel probe that delivers a series of short pulses of laser energy ('on') separated by rest periods ('off'). During the 'on' period the thermal energy acts on ciliary body epithelium, while during 'off' periods the adjacent structures are allowed to dissipate the heat, protecting them from the thermal effect. Therefore MP-TSCPC reduces the damage to the surrounding tissues and lowers the incidence of complications while preserving the IOP lowering activity.

This direction of research is reflected in the following published article:

Zemba M, Dumitrescu O, Vaida F, Dimirache E, Pistolea I, Stamate AC, Burcea M, **Branisteanu DC**, Balta F, Barac IR, Barac IR, et al: Micropulse vs. continuous wave transscleral cyclophotocoagulation in neovascular glaucoma. Exp Ther Med 23: 278, 2022 **IF 2.447**

https://doi.org/10.3892/etm.2022.11207

1.4.2. Aim

The review articles concerning the **uveal melanoma and the gene therapy in retinal diseases** provide an update and also discuss the current knowledge and research status regarding the diagnosis and treatment options in uveal melanoma and metastatic disease and the genetic manipulation techniques aimed at addressing visual impairment related to retinal disorders, both inherited and degenerative.

The primary aim of the study concerning the new **transscleral micropulse cyclophotocoagulation in the treatment of neovascular glaucoma** was to compare the performance of MP-TSCPC vs. CW-TSCPC over 12 months post-intervention. The secondary aim of the study was to demonstrate the safety and efficacy of MP-TSCPC over 12 months post-intervention.

1.4.3. Material and methods

For the **uveal melanoma diagnosis and current treatment options review**, the authors performed an extensive literature search in the Medline electronic database, using the PubMed interface. The keyword combinations used were 'uveal melanoma', 'iris melanoma', 'ciliary body melanoma', 'choroidal melanoma', and, in turn, each of the following: 'diagnosis', 'treatment', 'metastatic'. We included articles in English, published from January 1, 1990, to February 28, 2021. After filters were applied (case report, classical article, guideline, journal article, meta-analysis, observational study, review, systematic review) a consistent number of 3,012 references resulted. Among these, 93 references were cited in this review.

To assess the state of knowledge regarding **genetic therapy for retinal diseases**, the present study used MEDLINE/PubMed as the main biomedical database for research, using keywords, such as gene therapy, inherited retinal diseases, retinal dystrophies, viral

vectors, gene manipulation techniques, and age-related macular degeneration (AMD). From >800 articles, the authors selected a total of 35 studies for review, the majority of which were published between 2006 and 2020. The articles were selected based on their relevance for each subchapter taken into the discussion in the present review and also based on the elements of novelty brought by each study, as more recent ones were preferred for inclusion.

In order to evaluate the **comparative performance of MP-TSCPC vs. CW-TSCPC over 12 months post-intervention,** a retrospective cohort study was performed. Patients with NVG were treated with TSCPC between January 2017 and September 2019 at the Department of Ophthalmology of 'Dr. Carol Davila's Central Military Emergency University Hospital (Bucharest, Romania) was included. The study was not randomized, and the treatment modality (MP vs. CW) was not based on medical considerations related to the case. All patients followed a fixed postoperative visit schedule: day 1, day 7, months 1, 3, 6, 12, and 15. The primary time-point was 12 months post-intervention. The study was approved (approval no. 445/03.03.2021) by the Institutional Review Board of the hospital and followed the principles of the Declaration of Helsinki (2013).

The patient data were collected at baseline, before the TSCPC intervention on demographics of patients (age and sex), diagnosis including etiology of NVG, IOP, the number of glaucoma medications, including oral acetazolamide, best-corrected visual acuity (BCVA), anterior segment evaluation, and type of TSCPC used, MP or CW. A total of 51 eyes from 51 patients were treated, 27 with MP-TSCPC and 24 with CW-TSCPC. However, 5 were later excluded due to inadequate length of follow-up, and thus a remainder of 24 eyes for MP-TSCPC and 22 eyes for CW-TSCPC were included. The age was comparable between groups (P=0.45), with means of 55.6 years (range, 44-79) for MP-TSCPC and 58.1 years (range, 32-87) for CW-TSCPC. There were no differences between groups concerning sex (for example, males, 54.2 and 59.1% in the MP-TSCPC and the CW-TSCPC groups, respectively; P=0.97), as demonstrated in Table 1.9.

At each follow-up visit, IOP, BCVA, antiglaucoma medications, complications, and the need for retreatment were recorded. IOP was assessed by Goldmann applanation tonometry, or by I-care rebound tonometry when Goldmann applanation tonometry was not accurate or possible. BCVA was at an extremely low level, counting fingers (CF) or less. After the treatment, VA was divided into three groups, namely improved, unchanged, and worsened, compared with the baseline VA. A change in VA was defined as improved if it changed from light perception (LP) to perception of hand movements (HM) or improved, or from HM to CF, unchanged if it remained the same or worsened, when there was a decline in VA, either from CF to HM or LP or from HM to LP.

All procedures were performed in the operating room, under regional anesthesia; specifically, retrobulbar block with a mixture of 3 ml of lidocaine 4% and 1 ml of bupivacaine 1%. Transillumination was used when the position of the ciliary body was in doubt (high myopes, multiple surgeries on the anterior pole). Methylcellulose was used as a coupling agent, to facilitate the movement of the probe tip and to increase the laser power transmission.MP-TSCPC was performed with an MP P3 handpiece with the Iridex Cyclo G6 (IRIDEX Laser System). The power was set at 2000 mW and a duty cycle of 31.35% (micropulse 'on' for 0.5 msec and 'off' for 1.1 msec). The probe was applied using firm, moderate pressure in a continuous, sweeping motion over the superior and inferior quadrants, 90 sec for each hemiglobe. The 3 and 9 o'clock meridians, areas of scleral thinning, filtering blebs, and glaucoma drainage devices were avoided.

CW-TSCPC was performed with the G probe of Iridex Cyclo G6 (IRIDEX Laser System). A total of 75% of the eye circumference was treated. This usually required 6-7 applications in each quadrant, for a total of 20-21 shots. The initial power was 1,250 mW and the duration was 4 sec. The power of the laser was reduced by 200 mW if more than two 'pops' from disruption of the ciliary processes were heard. Postoperatively dexamethasone 0.1% every 6 h and cyclopentolate 1% twice daily were indicated. Patients continued their antiglaucoma therapy after the procedure. Therapy was later adjusted with the oral acetazolamide according to the IOP values recorded during the follow-up visits.

Table 1.9 - Patient characteristics at baseline.

Characteristics	Micropulse transscleral cyclophotocoagulation	Continuous wave-transscleral cyclophotocoagulation	P-value
Age (years)	55.6 (range, 44-79)	58.1 (range, 32-87)	0.30
Sex			0.74
Male	13 (54.2%)	13 (59.1%)	
Female	11 (45.8%)	9 (40.9%)	
Etiology of neovascular glaucoma			
Retinal vein occlusion	8 (33.3%)	8 (36.3%)	0.83
Diabetic retinopathy	9 (37.5%)	7 (31.8%)	0.69
Chronic uveitis	2 (8.3%)	1 (4.5%)	1.0
Chronic retinal detachment	2 (8.3%)	4 (18.9%)	0.41
Retinal artery occlusion	0	1 (4.5%)	0.49
Radiotherapy induced	1 (4.2%)	0	1.0
Carotid artery occlusive disease	2 (8.3%)	1 (4.5%)	1.0
Previous surgery			
Trabeculectomy	10	11	0.57
Iridectomy/iridotomy	3	2	1.0
Posterior vitrectomy	10	8	0.71
Cataract surgery	5	3	0.70
Ahmed valve implantation	1	1	1.0
Penetrating keratoplasty	0	1	0.49
Anti-VEGF injection	1.33 (range, 0-5)	0.95 (range, 0-5)	0.54
No previous surgery	8	7	0.91

Mean (range) or frequency (%) reported.

Patients were examined the following day, at one week, at 1, 3, 6, 9, 12, and 15 months. A minimum of 12 months of follow-up was required for study inclusion.

The primary outcome measure was a successful reduction of IOP: A 'favorable outcome' at any time-point was defined as post-procedural IOP ≤21 mm Hg or IOP reduction from baseline of ≥30%, with or without additional antiglaucoma medications. Hypotony was defined as an IOP of <5 mm Hg and was considered a failure of the treatment. Secondary outcome measures included the change in BCVA, the number of antiglaucoma medications, the necessity of oral acetazolamide, the complications, and the need for retreatment.

Baseline characteristics were summarized (number and proportions for categorical variables; mean and standard deviation for continuous variables) and compared between the two groups using Pearson's Chi-square test for categorical variables, and the Wilcoxon rank-sum test for continuous variables.

For the first study aim, the comparison of CW-TSCPC and MP-TSCPC procedures, the primary endpoint was the success of the intervention at 12 months. This was compared between the two arms using Pearson's Chi-squared test, and a 95%

confidence interval (CI95) of the difference in proportions of favorable outcomes between arms was reported. The proportion of favorable outcomes over time was compared between arms using longitudinal logistic regression using generalized estimating equations (GEE), which accounts for the correlation of outcomes within individuals. Moreover, the proportion of times with a favorable outcome over time between arms was compared using a one-degree of freedom Wald test within the GEE logistic regression model.

The secondary outcome was IOP, which was compared longitudinally between groups using a longitudinal general linear model (LGLM), including time treated as a factor, treatment, and their interaction. The model allows within-patient correlation and time-varying variance. The selection of the best correlation structure (unstructured, constant, exponential, and autoregressive of order 1), and time-constant vs. time-varying variance was made based on the Akaike information criterion. The comparison of the two treatment arms at month 12 used the Wald test of the LGLM.

The overall comparison of IOP trajectories over time between the two arms used the Wald test for the interaction in the LGLM. The important adverse events were considered worsening of VA, hypotony, and phthisis bulbi. The total of these three events was computed for each patient, and the rate of these events was compared between groups using a Poisson model, with a check for overdispersion (none was detected). Prevalence of individual important adverse events was compared between arms using Fisher's exact test. All analyses were conducted using the R statistical language and the 'nlme' package version 3.1-148 was used. $P<0.05$ was considered to indicate a statistically significant difference.

1.4.4. Results

Concerning the **ocular uveal melanomas,** they continue to represent an uncommon but potentially life-threatening ocular condition. They develop from melanocytes located in the highly pigmented uveal tract, the main oxygen, and nutriment provider of the retina. Anterior uveal melanomas originate in the iris while posterior uveal melanomas emerge from the choroid or the ciliary body. Among these tumors, choroidal melanoma is the most frequently diagnosed tumor (almost 90% of all uveal melanomas) followed by ciliary body melanoma (6% of the cases) and iris melanoma (4% of the cases) (Shields et al., 2009). Although uveal melanomas account for only 5% of all primary melanoma cases (90% located in the skin), they represent the most frequently diagnosed primary intraocular malignant tumor in adults.

While the incidence of cutaneous melanomas has continuously increased, the incidence of uveal melanomas has remained constant in the last decades across all continents. However, there are consistent differences in incidence between different areas worldwide (Singh et al., 2011). In the US, the incidence varies from 5.1 to 6 cases per million population per year, being highest in the southern latitudes. In Europe, the incidence of uveal melanomas is much higher (up to 8 cases per million population per year) in Caucasians of northern European descent (Scandinavia and Baltic States) and significantly lower in Italy (3.3 cases per million population per year), and Spain (1.9 cases per million population per year) (4). Hispanics and Asians have a lower incidence while Black individuals have the lowest one. The relative risk of uveal melanoma has been estimated to be 1/1.2/5/19 in Blacks/Asians/Hispanics/Non-Hispanics, respectively (Hu et al., 2005). There is no consistent sex-related difference; still, in epidemiological studies, the age-adjusted incidence has revealed that men have an increased predominance (5.8 per million in males as compared to 4.4 per million in females). Uveal melanomas

are uncommon in children. While the mean age for diagnosis has increased from 55 years of age to 62 years of age in Caucasians, in Asian countries uveal melanoma seems to appear at a younger age (Tan et al., 2020). Therefore, the mean age for diagnosis varies from 45 years of age (in the Chinese population) to 55 years of age (in the Japanese population).

To date, there are several risk factors identified in uveal melanoma development. Host susceptibility factors such as light-colored eyes, fair skin color, dysplastic nevus syndrome, ocular melanocytosis, and xeroderma pigmentosum have been confirmed as predisposing factors (Shields et al., 2013). In particular, a pre-existing iris or choroidal nevus is of major concern as they can evolve into melanoma. The continuous nevus growth, together with the appearance of ectropion uveae and/or spontaneous hyphema (in the case of iris nevus) and subretinal fluid and orange pigmentation (in the case of choroidal nevus) highly suggests a transformation into melanoma (Shields et al., 2009). Excessive exposure to natural and artificial ultraviolet light and also to blue light has been suggested as risk factors (Shah et al., 2005). Patients with BRCA1-associated protein 1 (BAP1) mutation are considered to have a higher risk of developing uveal melanomas at a younger age (Harbour et al., 2010). Most uveal melanomas have mutations in the G protein subunit alpha Q (GNAQ) or G protein subunit alpha 11 (GNA11) genes (90%) (Van Raamsdonk et al., 2009) and in the phospholipase C β4 (PLCB4) and the G-protein coupled receptor cysteinyl leukotriene receptor 2 (CYSLTR2) (Johansson et al., 2016, Moore et al., 2016). In metastatic disease, there is a loss of chromosome 1p that leads to higher mortality when accompanied by the concurrent loss of chromosome 3(Kilic et al., 2005).

Iris melanoma is incidentally diagnosed at slit-lamp evaluation, usually much earlier (10 to 20 years) than other uveal melanomas. Sometimes, the patient solely notices the iris color changes (heterochromia). In most cases, the tumor is circumscribed, located inferiorly, and induces pupillary distortion (corectopia). Ectropion iridis, hyphema, secondary glaucoma, cataract, and extraocular extension are the most frequent complications. The intraocular pressure rise is the consequence of trabecular meshwork invasion or direct angle compression. The diagnosis of diffuse iris melanoma is more challenging and is often delayed due to the infiltrative pattern. Ring iris melanoma is a rare entity with angle location often simulating unilateral pigmentary glaucoma (Conway et al., 2001). The current T1-T4 classification, with further subgroups of iris melanoma, according to the American Joint Committee on Cancer Classification (AJCC), is based on tumor size (clock hours), tumor extension (ocular and extraocular), and complications (glaucoma) (Kivela et al., 2017). While ultrasound biomicroscopy (UBM) and anterior segment optical coherence tomography (AS-OCT) are helpful tools in evaluating small anterior tumors, the B-scan ultrasonography can better evaluate larger tumors with posterior extension. Fine needle aspiration biopsy (FNAB) confirms the diagnosis (Russo et al., 2020).

The diagnosis of posterior uveal melanoma is usually performed by an experienced clinician during a routine slit-lamp biomicroscopy and/or indirect ophthalmoscopy under dilated pupil, as many tumors (especially ciliary body tumors) can silently grow for years and are asymptomatic (up to 40% of the cases). Most frequent complaints include floaters and photopsia. While tumors located in the periphery can reach consistent dimensions until visual field loss is noted, in locations closer to the macula and the optic disc, the cystoid macular edema or secondary retinal detachment induces prompt visual loss. The funduscopic evaluation typically reveals a pigmented dome-shaped nodular mass, well-circumscribed, located under the retinal pigment epithelium. The degree of pigmentation can largely vary. In partially pigmented (30% of

the cases) and amelanotic tumors (15% of the cases), the abnormalities of overlying retinal pigment epithelium and also the tumor vascularization can be easily observed. An accompanying massive exudative retinal detachment can hide the diagnosis. A mushroom-shaped aspect highly suggests that the Bruch membrane has been surpassed and is noted in 20% of the cases. Rarely, larger tumors induce vitreous hemorrhage. Tumors located anteriorly show dilated overlying episcleral vessels ('sentinel vessels'), secondary glaucoma due to the anterior iris-lens diaphragm displacement or tumor extension into the angle, and cataract. Severe ocular pain due to posterior ciliary nerve involvement, transscleral tumor growth under the conjunctival, and proptosis due to orbital extension have also been reported (Kaliki et al., 2017).

According to the Collaborative Ocular Melanoma Study (COMS), choroidal melanoma is currently classified as small (largest basal diameter ≤16 mm, apical height between 1.5 and 2.4 mm), medium-sized (largest basal diameter ≤16 mm, apical height between 2.5 and 10 mm), and large (largest basal diameter ≤16 mm, apical height ≥10 mm). The American Joint Committee on Cancer (AJCC) has updated the staging system according to size criteria (T1-T4), ciliary body involvement, and episcleral extension (Finger et al., 2009).

There is a consistent improvement in uveal melanoma diagnosis accuracy in the last decades, from around 20% to more than 99%, as indicated by the COMS report. The 10 MHz B-scan ultrasonography is an essential evaluation tool in ocular oncology, easily revealing tumors with a thickness of more than 1.5 mm. It is particularly useful in opaque intraocular media. On B-scans, uveal melanomas have different shapes (collar-stud or mushroom appearance), have a low to moderate internal reflectivity, present a choroidal excavation, and can be accompanied by a secondary retinal detachment. This technique is essential for measuring tumor dimensions, evaluating the extent, planning the treatment, and follow-up. The 40 MHz anterior UBM can visualize anterior uveal melanomas and differentiate them from those originating in the ciliary body. On A-scan there is a highly reflective anterior border followed by decreased amplitude as the tumor mass is acoustically hollow (positive kappa angle), and a significant final echo. Transillumination, which is particularly helpful in finding tumor borders, has a limited precision in partially pigmented and amelanotic tumors (Kaliki et al., 2017). Fluorescein angiography (FA) and indocyanine green angiography (ICG) offer no pathognomonic signs in uveal melanomas. They have a limited contribution in differentiating choroidal nevi from small tumors but they can reveal, in larger tumors, a patchy fluorescent pattern (in FA) and the internal tumor vascularization known as the 'double circulation pattern' (mainly in ICG). Fundus autofluorescence due to lipofuscin pigmentation is more intense than the autofluorescence of drusen usually seen in choroidal nevi. Orbital computed tomography (CT) and magnetic resonance imaging (MRI) with contrast are less sensitive diagnostic tools but help detect the extrascleral extension and in differentiating choroidal melanoma (which is enhanced by contrast) from choroidal detachment (no contrast enhancement) or choroidal osteoma (calcium detection). The Color-Doppler ultrasound can differentiate the tumor from choroidal nevi due to the presence of a typical pulsatile blood flow at the tumor base. While regular spectral-domain OCT has limitations in accessing the tumor's internal structure, the newer enhanced depth imaging spectral-domain OCT can see deeper into the choroid and reveal the tumor, the thinned choriocapillaris, the accompanying retinal fluid, retinal changes, and retinal deposits (lipofuscin). An incisional biopsy is an invasive diagnostic tool involving the risk of complications and cancerous cell spreading and is currently indicated in uncertain cases only, such as amelanotic tumors (Singh et al., 2012).

Fine-needle aspiration biopsy (FNAB) may soon become a standard procedure in conservatively treated melanomas as it provides the samples mandatory for genetic analysis with direct implications for prognosis and metastasis rates (Onken et al., 2012). Thus, according to gene expression profiling (GEP), ocular melanomas have been subdivided into 2 types. Class 1 tumors (further subdivided into class 1a and 1b), representing almost 50% of the cases, have a low and intermediate metastatic risk at 5 years (2 and 21%, respectively), while class 2 tumors have a significantly higher risk (72%) (Onken et al., 2010). The histological evaluation of the enucleated eye reveals 3 types of tumor cells: spindle A, spindle B, and epithelioid. The epithelioid has frequent mitotic figures, and morphologic variations and is highly anaplastic. Thus, epithelioid cell melanoma and mixed cell melanoma are considered to have a significantly poorer prognosis as compared to spindle cell melanomas and necrotic melanomas.

Overall, iris melanoma has a better prognosis and a lower mortality rate as it develops metastases in only 2 to 7% of the cases, higher (10%) if there is mixed cellularity or ciliary body involvement. Choroidal melanoma has a much higher rate of metastasis (in almost 50% of the cases), mainly hematogenously, involving the liver (90%), the lungs, the brain, the kidneys, and the bones (Diener-West et al.,2005). The subsequent life expectancy is limited from 6 to 12 months (Krantz et al., 2017). For many years, the tumor size, epithelioid type, ciliary body or optic nerve involvement, and extrascleral extension were considered the main indicators for metastasis and mortality. Currently, the specific genetic profile (chromosome 3 deletion, chromosome 8q gain, BAP1 loss, chromosome 1p, and 9q loss, Class 2 GEP) seems to be a better indicator for metastatic disease (Kaliki et al., 2015). Liver enzyme levels and chest X-rays should be routinely performed at the time of diagnosis to rule out the most frequent concomitant liver and/or lung metastasis (Singh et al., 2018).

The therapeutic attitude in primary uveal melanoma largely varies mainly according to the tumor size. Usually, small uveal lesions are closely monitored clinically and with sequential photography. While UBM is a helpful adjunct in monitoring iris lesions, B-scan ultrasonography is mandatory to detect any signs of growth of posterior lesions. In the literature, iris nevi have a transforming rate estimated at 5% in 5 years and 11% in 20 years (Territo et al., 1988). On a larger series, the transformation rate was 2% at a mean follow-up of 5.6 years and 8% by 15 years and a systematical evaluation of predictive factors according to the mnemonic ABCDEF (A, age younger than 40 years; B, blood (hyphema); C, clock hour inferiorly (location); D, diffuse flat configuration; E, ectropion uveae, and F, feathery margins) was proposed to simplify the early detection of iris nevus growth into melanoma (Shields et al., 2013).

Choroidal nevi evolution must also be carefully monitored. Usually, choroidal nevi are a chronic condition easily recognizable by the accompanying drusen and pigment epithelium atrophy on the surface and by the nonpigmented surrounding halo. They are detected in about 6% of the Caucasian population and have an annual conversion rate into melanoma that increases with age, estimated between 1 in 5,000 and 1 in 8,800 cases (Singh et al., 2005, Shields et al., 2000). Nevertheless, the appearance of visual symptoms, subretinal fluid, increasing thickness over 2 mm, orange pigmentation, absence of drusen and surrounding halo, ultrasound hollowness, and margin touching the optic disc are considered indicators for tumor conversion. Like in iris melanoma, the mnemonic TFSOM UHHD (‘To Find Small Ocular Melanoma Using Helpful Hints’), derived from ‘Thickness, Fluid, Symptoms, Orange pigment, Margin, Ultrasonographic Hollowness, Halo absence, and Drusen absence’ was created by Shields et al to help practitioners to better evaluate the ocular melanoma risk factors. A careful evaluation of these features is of particular importance as the chance for tumor growth at 5 years is

around 3% when no risk factors are encountered and over 50% when two or more factors are noted. Prospective cohort studies suggest that early treatment is better than observation, in some patients, for preventing death from metastatic disease (Damato et al., 2014, Straatsma et al., 2003). In the particular case of elderly patients with active tumors, but with consistent comorbidities restricting therapeutic options or very low life expectancy, observation is a feasible choice.

Enucleation, once the gold standard in the treatment of intraocular tumors, is still indicated in large uveal melanomas (>18 mm in basal diameter and >12 mm in thickness), in cases with total visual loss due to severe complications, and tumors refractory or recurrent to conservative treatments. Eyes with advanced orbital tumor extension are currently treated more conservatively avoiding orbital exenteration by combining enucleation with local radiation therapy (Jager et al., 2020). The Zimmerman-McLean-Foster hypothesis that enucleation accelerates mortality has been ruled out (Singh et al., 2004). Nevertheless, enucleation must be carefully performed as any excessive manipulation or injury to the affected eye during surgery carries the risk of tumor cells spreading into the bloodstream and orbital tissue, as suggested by the occurrence of orbital recurrences after enucleation. Performing external radiation before enucleation does not change the 10-year survival rate in large choroidal melanoma as compared to enucleation alone. The superiority of enucleation over conservative iodine-125 brachytherapy in reducing the risk of metastasis in medium-sized tumors has not been confirmed by the Collaborative Ocular Melanoma Study (COMS) in up to 12 years of follow-up.

Small iris melanoma is often successfully managed by sector iridectomy, especially if the tumor induces secondary glaucoma or interferes with the vision. Iridocyclectomy is preferred in rapidly growing iris tumors involving the angle (Rospond-Kubiak et al., 2014). Sclerouvectomy (transscleral resection) is a full-thickness excision including the sclera, the tumor, the choroid, and the retina. Usually, the scleral excision is around 3 mm larger than the melanoma. While banked sclera is mandatory for reconstruction, adjacent transscleral cryotherapy is necessary for retinal stability. Lamellar sclerouvectomy is less invasive to the retina than the previous technique (due to the partial-thickness scleral flap) but it carries a much higher risk for tumor reoccurrence (Shields et al., 1991). The most frequent complications of surgical excision are retinal detachment, vitreous hemorrhage, incomplete tumor removal, and cataract. Tumor endoresection during pars-plana vitrectomy is technically feasible but requires vast experience as the tumor margins are not always clearly visible. Although there is a major concern about the intraocular and extraocular tumor spread during surgery, a study found a lower rate of metastatic spread during endoresection (3.7%) as compared to the iodine 125 brachytherapy group (20.4%) (Caminal et al., 2013). Nevertheless, the addition of local radiotherapy reduces the reoccurrence rate (Biewald et al., 2017, Susskind et al., 2017).

Conservative treatment using a radioactive plaque temporarily sutured on the sclera adjacent to the tumor is one of the oldest (since the 1980s), most effective, and most widespread methods for controlling medium-sized posterior uveal melanomas (Brewington et al., 2018). It is also effective in iris melanoma treatment, but it has a significantly higher rate of cataract formation and eyelid scarring. The plaques have different sizes and shapes and must exceed the largest basal diameter of the tumor by 2 mm (Dogrusoz et al., 2017). The intraoperative usage of transillumination is mandatory to adequately temporarily attach to the sclera the plaque that will be removed after 3-7 days. Different isotopes with good tissue penetration are used to radioactively charge the plaques. Among them, ruthenium 106 is most frequently used in Europe while in the US

iodine 125 is preferred. The usage of cobalt 60, iridium 192, palladium 103 and strontium 90 is less frequent (Weis et al., 2016). The time and the amount of energy delivered are calculated according to the tumor size. Thus, efficient, customized irradiation is performed at both the tumor base and apex with minimal adjacent tissue involvement. Brachytherapy is highly effective in tumor destruction so most of the treated melanomas show a consistent regression or even total flattening (Fallico et al., 2021). While the tumor resistance rate widely varies between different centers, the local recurrence rate after iodine 125 or ruthenium 106 brachytherapy (in a diffuse manner or starting from the initial tumor margins) is estimated at 9.6% of cases and indicates the need for enucleation (Chang et al., 2013). Second double-dose brachytherapy has shown long-term efficacy in further decreasing or stabilizing the tumor size thus reducing the need for enucleation (Grange et al., 1999). A Swedish long-term patient survival study after plaque ruthenium 106 brachytherapy performed between 1980 and 1999, revealed excellent relative survival rates (97% at 1 year, 74% at 5 years, 64% at 10 years, 64% at 15 years, 62% at 20 years, 70% at 25 years, 83% at 30 years, 114% at 35 years and 200% at 40 years) and that 82% of uveal melanoma-related deaths occurred in the first decade after treatment (Stalhammar et al., 2020). A retrospective evaluation of small choroidal melanomas treated with iodine 125 between 2004 and 2017 also showed that, after 3 years of follow-up, the survival rate was 97% with no metastatic events and that 69% of the patients retained visual acuity of at least 20/50 (Yupari et al., 2021). Unfortunately, brachytherapy carries the risk of local complications such as cataract formation, scleral necrosis, neovascular glaucoma, and dry eye (Detorakis et al., 2005). Radiation retinopathy is dependent on radiation dose and can occur in around 30% of the treated patients within 2 years of treatment (Krema et al., 2009). Radiation maculopathy and in particular radiation-induced macular edema can be efficiently controlled with intravitreal anti-vascular endothelial growth factor treatment or intravitreal dexamethasone implants (Fallico et al., 2021). Radiation optic neuropathy is responsible for irreversible, sudden onset, visual loss within years after treatment of a tumor mainly located near the optic disc, most probably due to irreversible local vascular damage (Indaram et al., 2015).

Proton beam radiotherapy was developed in the early 1990s. The charged-particle radiation is a newer conservative treatment, using protons or helium ions as charged particles to more precisely and much more safely deliver the desired amount of energy in different tumor parts. This method is a frequent conservative alternative to brachytherapy or enucleation for the treatment of unresectable or diffuse iris melanoma and in medium-sized or larger posterior melanomas if a charged-particle accelerator is available. The treatment is usually fractionated and is preceded, in the case of posterior uveal melanomas, by the initial scleral suture of tantalium rings that serve as radiopaque tumor reference markers. During the irradiation sessions, the head and eye must be carefully positioned. Although the efficacy seems to be similar to brachytherapy (Phillips et al., 2013), there are major advantages due to more homogenous and focused treatment and less damage to the surrounding tissue (Gragoudas et al., 1999). Still, radiation-associated complications can occur in time in almost 50% of the cases. Similar to brachytherapy, most of the tumors stop growing or regress after treatment. Also, the enucleation and the recurrence rates after treatment are comparable. The survival rate after charged-particle irradiation is comparable to that after enucleation (Gragoudas, 2006, Papakostas et al., 2017).

A recent meta-analysis of gamma knife radiosurgery as a primary treatment option in more than 1,000 uveal melanoma cases in the last 5 decades has shown that gamma knife radiosurgery is efficient in controlling the tumor in 96% of the cases with a 5-year survival rate of 76%. Still, further comparative randomized studies are needed to evaluate

the position of this technique in the current therapeutic armamentarium (Parker et al., 2020).

Direct laser photocoagulation of the uveal melanoma was the first conservative method introduced by Meyer-Schwickerath in the early 50s. Today, this technique, with limited indication on small tumors only located at a distance from the fovea, is abandoned in many centers due to the modest tumor control and the increased rate of recurrence. Moreover, direct laser photocoagulation is associated with an increased risk of tumor extension through the Bruch's membrane, choroidal neovascularization, macular edema, retinal tractions and detachment, and vitreous hemorrhage (Foulds et al., 1986).

Transpupillary thermotherapy (TTT) uses a near-infrared diode laser. The local rise in temperature slightly over 45˚C offers better results than direct photocoagulation in the control of small melanomas and a lower tumor reoccurrence rate, especially when used in conjunction with brachytherapy. In a retrospective evaluation of primary TTT in choroidal melanoma, the tumor reoccurrence rate in a 2001-2012 group was 11% at 5 years and 15% at 10 years, significantly lower than in the previous group treated between 1995 and 2000 (Mashayekhi et al., 2015). Complications after TTT are noted in 44% of the cases and include retinal vascular occlusions, cystoid macular edema, epiretinal membranes, vitreous hemorrhage, optic disc atrophy, retinal traction and detachment (Chojniak et al., 2011).

Photodynamic therapy (PDT) using verteporfin as a photosensitizer has been FDA approved in ophthalmology since the 2000s for the selective treatment of choroidal neovascularization secondary to various conditions due to minimal surrounding destruction. Primary PDT was found to be followed by complete tumor regression in 67% of small amelanotic choroidal melanomas at a 5-year follow-up with no significant side effects on macular or optic nerve function (Turkoglu et al., 2019). A recent meta-analysis of published studies found an overall 80% response rate to treatment, especially in small amelanotic tumors (Yordi et al., 2021). While these results suggest that PDT is an effective primary treatment for small choroidal melanoma, especially in cases without pigmentation, further long-term studies are required for validation.

Although the metastatic disease is detected in less than 1% of the patients with uveal melanoma at the time of initial diagnosis (Diener-West et al., 2004), a significant percentage of these patients will develop time metastatic disease (31% of cases within 5 years, 45% within 15 years, and almost 50% within 25 years) (Kujala et al., 2003). The dramatic decline in survival rate from 70% at 5 years for the primary disease to only 8% at 2 years after metastatic disease as reported (Damato et al., 2011) confirms the need for an urgent treatment regimen and appropriate psycho-oncology support in such cases.

The usage of systemic chemotherapy has offered poor results suggesting that uveal melanoma is resistant to current chemotherapies. Conventional drugs (dacarbazine, temozolomide, and fotemustine), and many of the modern agents (paclitaxel, docosahexaenoic acid, and liposomal vincristine) have offered discouraging results, in monotherapy and also in combination. The most encouraging data have been noted with the combination of treosulfan and gemcitabine which showed a median survival of 14 months and an annual survival rate of 80% (Pfohler et al., 2003). Still, due to the frequent hematological, neurological, and pulmonary adverse effects that consistently lower the quality of life in these patients, systemic chemotherapy has not been routinely implemented for the treatment of metastatic disease.

Chemoimmunotherapy has also limited efficacy in uveal melanoma. The immune privilege of the eye may explain why promising preliminary results of recombinant interferon α-2b are associated with the BOLD regimen (bleomycin, vincristine, lomustine, and dacarbazine) were not confirmed (Nathan et al., 1997).

While immunotherapy alone using different agents (ipilimumab, pembrolizumab, or nivolumab) has shown limited results, the combination therapy (of ipilimumab with one of the previously mentioned agents) has offered encouraging results with an overall survival rate of around 19 months (Pelster et al., 2021, Najjar et al., 2020). The side effects were found to vary from easily manageable skin reactions and pseudo-flu symptoms to more serious autoimmune colitis and thyroid and pituitary hormonal alterations. A consistent number of phase I and II clinical trials are currently underway to evaluate the efficacy and safety of novel immune-based therapies (such as cell-based and peptide vaccines, adoptive transfer of autologous TILs or CAR-T cells directed against human epidermal growth factor receptor 2), or different therapeutic combinations (pembrolizumab and entinostat, ipilimumab and melphalan PHP, ipilimumab and laparoscopic radiofrequency ablation) (Carvajal et al., 2017).

Molecular-targeted therapy seemed to be a suitable approach for uveal melanoma due to the distinctive genetic profile, with mutations in the GNAQ and GNA11 genes stimulating cell proliferation (Carjaval et al., 2017). Unfortunately, several mitogen-activated protein kinase (MAPK) pathway inhibitors (used alone or in combination with chemotherapy) and also heat shock protein 90 inhibitors have failed to exhibit significant efficacy in clinical trials (Piperno-Neumann et al., 2014).

In regards to hepatic metastasis, liver-directed therapies, including intra-arterial chemotherapy with fotemustine, transarterial liver chemoembolization, and isolated hepatic perfusion have shown, besides their significant theoretical advantages, encouraging results in different studies (Mallone et al., 2020, Artzner et al., 2019). Different surgical laparoscopic excisions (alone or combined with radiofrequency ablation), liver radioembolization (using yttrium-90 microspheres), and liver thermotherapy have also shown promising results and are under evaluation (Mariani et al., 2009, Gonsalvez et al., 2019, Rodriguez-Vidal et al., 2020).

Regarding **gene therapy for retinal diseases,** the literature reveals a continuously progressive trend over the last two decades with promising advancements in the treatment of inherited, previously intractable diseases. Gene therapy techniques have in common the insertion of a foreign DNA fragment into host cells, aiming to modify the expression of proteins by the target cells. The delivery of the genetic material inside the target cell can be achieved by viral or non-viral methods. As viruses naturally infect human cells and insert their genetic material into the host cell nucleus, they can be considered a very efficient vector for genetic manipulation. The pathogenic viral genes are removed and the virus is used to insert the therapeutic gene inside the target cell. Lentiviruses and retroviruses can integrate their genetic material directly into the host genome. Herpes viruses and adenoviruses insert their genetic material as extrachromosomal episomes (Yáñez-Muñoz et al., 2006).

Lentiviruses have a single strand of RNA and are able to integrate their genetic material inside the chromosomes of the host, thus being capable of replicating continuously following a single administration, even in dividing cells, and sustaining long-term and stable transgene expression, even for large genes up to 10 kb. A possible disadvantage in using lentiviruses for gene delivery is insertional mutagenesis, which may lead to the alteration of different genes with either the compromising of the cell's viability or the continuous replication and formation of a tumor.

Adenoviruses are double-stranded DNA viruses, which as vectors are able to transport the largest amount of genetic material of any viral vectors, up to 37 kb. These viruses can infect dividing and non-dividing cells as well; however, their genetic material remains in the episomes, and thus the risk of insertional mutagenesis is significantly

lower. Adenoviruses 2 and 5 are the most frequently used serotypes, which can transduce cells of the retinal pigment epithelium (RPE) cells and in some cases, photoreceptors. However, the disadvantage is that due to the lack of integration of the genetic material into the target cell genome, the genetic information inside the episomes is diluted with each mitosis cycle of dividing cells. Another drawback is the important immune response stimulated by the adenoviruses inside the organism of the host, which can remove all host cells that express adenovirus proteins via cytotoxic T-cell intervention, thus also eliminating the genetic information required for transduction. To elude the host immune response, helper-dependent adenoviruses have been developed (Parks et al., 1996).

In ocular gene therapy, adeno-associated viral vectors are currently widely used. These are small and non-enveloped DNA viruses that belong to the Parvoviridae family and are non-pathogenic as they require a helper virus for replication. Similar to adenoviruses, they can infect both dividing and non-dividing cells and their genetic material remains inside the episomes. The capacity of adeno-associated viral vectors for packaging genetic material is only 4.8 kb and certain large genes cannot be inserted inside their genome. Serotypes 1, 2, 4, 5, 6, 7, 8, and 9 have tropism for the retinal tissues and are able to transduce cells of the RPE; however, the most frequently used serotypes in subretinal delivery are 2, 5, and 8. Recombinant vectors can be used, which are hybrid or pseudo-typed adeno-associated viruses (AAVs), meaning they have components from various serotypes. Due to this characteristic, they are able to elude the immune response and have a higher transduction efficiency and an increased cellular tropism. Pseudo-types 2/1, 2/4, and 2/6, which recombine components from the correspondent serotypes, are efficient in transducing cells of the RPE, while pseudo-types 2/5, 2/7, 2/8, and 2/9 are most efficient in the transduction of photoreceptor cells (Manfredi et al., 2013, Schon et al., 2015).

Over the past decade, second-generation vectors were introduced to ocular gene therapy. These are viruses that have a modified capsid structure, in order to prevent its degradation, increase tropism for specific cells, or to remove the antibody binding sites. Another category of second-generation vectors has a directed evolution, based on the accumulation of mutations, similar to natural selection, from which are selected the mutations that are proven to increase the transduction efficacy or render the vector capable of crossing biological barriers.

In order to increase cellular specificity, promoters and enhancers may be used. Following the integration of the genetic information brought to the target cell by the viral vectors, promoters, such as cytomegalovirus, allow the transcription of the desired transgene only in some specific cells. Thus, it is possible to target only the cells of the RPE or photoreceptors, while non-target cells will not transcribe the genetic material inserted by the vector, in the absence of promoter recognition (Dalkara et al., 2013, Mace et al., 2015).

Non-viral methods consist of chemical and physical techniques of introducing the DNA inside the nucleus. Although they are less immunogenic, they are also less efficient in targeting cells in vivo. However non-viral techniques can deliver large genes into the host genome, they may be repeated and a larger dose may be used. Lipid- or polymer-based carriers are used in non-viral genetic manipulation and as these systems are not self-replicating, the administration needs to be repeated.

Liposomes consist of amphiphilic molecules, such as cholesterol and phospholipids, which can merge with the cellular membrane that also has a double-layered phospholipidic structure. Solid lipid nanoparticles have a lipid core surrounded by a layer of surfactants in an aqueous dispersion and may vary in size between 50 and 1,000 nm, which renders it possible for them to be used in a subretinal injection delivery

method. Bioerodible polymers, such as polylactic-co-glycolic acid) (PLGA), polyesters [poly(lactic acid); PLA], hyaluronic acid, and chitosan are under investigation as possible carriers for genetic therapy (Kiss, 2019, Ramamoorth et Narvekar, 2015, Pitkanen et al., 2003).

The most commonly used delivery routes for the novel genetic information towards the retinal tissue with the aid of viral vectors are intravitreal injection, surgical subretinal injection, and suprachoroidal administration. Intravitreal injections are one of the safest and most widely used methods for therapy delivery in ophthalmology, particularly in the treatment of retinal diseases. When used to deliver genetic information to the retinal ganglion cells with the aid of AAVs, the intravitreal injections are not very efficient due to the presence of the vitreous and the internal limiting membrane. Vitrectomy and chemical-induced vitreous detachment by microplasmin, have been proved to aid vector penetration in retinal tissue (Ivanova et al., 2010). The need for vitrectomy transforms the procedure into a surgical intervention. For this reason, an intravitreal injection for gene delivery is used when targeting the inner retinal layers or the retinal ganglion cell layer, as in Leber hereditary optic neuropathy (Tshilenge et al., 2016). In order to deliver genetic information to the cells of the RPE or photoreceptors, a subretinal injection is preferred. This method became the most frequently used for genetic therapy in ophthalmology, particularly following the approval of Luxturna by the FDA (Darrow, 2019). After a complete vitrectomy is performed, a 41 G soft-tipped cannula is used to reach the subretinal space. The therapeutic substance may be injected directly or it may be injected after first creating a subretinal balanced salt solution (BSS) bleb, which aids in the hydrodissection of the subretinal space, but can dilute the drug. The procedure is completed by a fluid-air exchange. Inside the bleb, there is a high concentration of vector that enters the available cells, thus inducing a high expression of the desired transgene. However, cells situated outside the bleb will express a low amount of the transgene. Another possible disadvantage of this method of delivery may be the damage to the photoreceptors due to the separation between the RPE and the photoreceptor outer segments, particularly in the foveal area. A subretinal injection may be difficult in eyes with subretinal fibrosis, which may lead to the appearance of macular holes, due to the high injection pressure (Davis et al., 2019). The suprachoroidal administration is a less invasive procedure that delivers the therapeutic substance to a virtual space between the sclera and the choroid. The procedure has been proven to be safe and effective when using a triamcinolone injection, with the aid of microneedles (Campochiaro et al., 2018). In gene therapy, suprachoroidal delivery has been used in preclinical studies. In a previous study, An AAV8 vector that expresses a vascular endothelial growth factor (VEGF)-neutralizing protein, termed RGX-314, was proven efficient in suppressing the vascular leakage associated with vascular disease in a rat model. The transgene expression level was similar to that obtained with the subretinal injection. However, the various AAVs exhibited differential efficacy in inducing transgene expression, which may be an effect of anti-AAV antibodies present in the host organism (Ding et al., 2016).

Gene therapy for inherited retinal diseases aims to replace a defective gene that is causing the illness with a normal one, delivered either in vivo or ex vivo, through cultured cells. The eye is an effective target for in vivo delivery, aided by viral vectors, as it is an immune-privileged organ. Current research on gene therapy for inherited retinal diseases is based on the gene augmentation technique. Following insertion, the normal gene is present in the episomes, inside the extrachromosomal DNA and the original, defective gene is still present. This approach is effective for inherited diseases that are associated with loss-of-function mutations (DiCarlo et al., 2018).

The first gene therapy for an inherited retinal disease, approved in 2017, was voretigene neparvovec-rzyl (Luxturna, Spark Therapeutics) for retinal dystrophies with RPE65 mutations. If both RPE65 alleles present pathogenic mutations, patients develop autosomal recessive Leber congenital amaurosis or retinitis pigmentosa, presenting with progressive and severe visual loss, commencing from childhood. The RPE65 gene is expressed in the RPE and it encodes for RPE-specific 65 kDa protein, an enzyme necessary for converting 11-trans-retinil, which is not photoactive, to 11-cis-retinal, used by the photoreceptors during the visual cycle for visual pigment synthesis and regeneration (Tang et al., 2011, Miraldi et al., 2018). Luxturna is injected into the subretinal space and it involves the use of an AAV2 for the delivery of a human RPE65 to the cells of the RPE. This therapy has been shown to lead to an improvement in night vision, visual field, and light sensitivity (Gao et al., 2020).

The clinical manifestations of pigmentary retinopathy are linked to the retinitis pigmentosa GTPase regulator (RPGR) gene mutation, which is involved in the regulation of ciliogenesis in photoreceptor cells. The loss-of-function mutation of the RPGR gene leads to the degeneration of the photoreceptors and the occurrence of either pigmentary retinopathy, cone dystrophy, or cone-rod dystrophy. Three early-phase clinical studies are evaluating the efficacy of gene therapy in these photoreceptor dystrophies (ClinicalTrials.gov Identifier: NCT03116113, NCT03252847, and NCT03316560). The vectors used in these trials are AAV2 or AAV2/5 associated with rhodopsin kinase promoter or AAV8 associated with codon-optimized RPGR (Martinez-Fernandez et al., 2018).

Another inherited retinal disease targeted for gene therapy is the X-linked retinoschisis. In this case, the trigger of the pathogenic mechanism is the loss of function of the retinoschisis 1 (RS1) gene, expressed by the photoreceptors and bipolar cells, which plays an important role in the maintenance of retinal structural integrity. Visual loss begins in childhood and it is caused by macular schisis, retinal detachment, and vitreous hemorrhage. Two studies have been published using AAV and different gene variants, which are injected into the vitreous cavity (Cukras et al., 2018, Mishra et al., 2021).

An AAV2 vector linked to a cone opsin promoter, which is administered via subretinal injection was used in a recent trial to induce the expression of the cyclic nucleotide-gated cation channel alpha-3 (CNG)A3 or CNGB3 gene in achromatopsia. The loss-of-function mutations of these genes generate the clinical manifestations of complete or incomplete achromatopsia. In complete achromatopsia, patients present with nystagmus, photophobia, a complete lack of color discrimination, and loss of central vision from birth. These symptoms are less severe in incomplete achromatopsia (Michalakis et al., 2017).

In choroideremia, which is an X-linked inherited retinal disease, the defective CHM gene encoding for Rab escort protein 1 (REP-1) protein determines the loss of function of this protein, resulting in progressive RPE, choroidal and retinal atrophy. The loss of peripheral vision and nyctalopia begin in early childhood. A phase 2 (NCT02341807) and a phase 3 study (NCT03496012) is undergoing, both using AAV2 in order to deliver the CHM gene via subretinal injections, with both showing a good safety profile. In addition, a phase 1 study by 4D Molecular Therapeutics (NCT04483440) is being conducted and is studying the efficacy of an intravitreal injected AAV carrying a transgene (Lam et al., 2019).

Mutations of the ATP binding cassette subfamily A member 4 (ABCA4) gene stand at the base of the clinical features of Stargardt disease. This macular dystrophy causes progressive central visual loss, difficulties in dark adaptation, and color vision

loss. The ABCA4 gene, which is a large gene that cannot be contained in AAV vectors, encodes for both a membrane transporter implicated in phototransduction and for the removal of metabolites that result from this process. A trial sponsored by Sanofi uses an equine anemia lentivirus, StarGen, incapable of causing disease in humans, in order to transport the genetic material to the host cells. The results revealed that StarGen was well-tolerated following subretinal injection (Binley et al., 2013).

Usher syndrome associates pigmentary retinopathy, congenital hearing loss, and vestibular dysfunctions, caused by mutations in the myosin VIIA (MYO7A) gene. The MYO7A gene is also large and cannot be transported by AAV vectors, which is why the same lentivirus as for the Stargardt disease trial is used, via subretinal injection (Zallocchi et al., 2014).

Gene therapy may represent an alternative to anti-VEGF therapy also in age-related macular degeneration (AMD). Ongoing trials are assessing the possibility to treat AMD, which is one of the most common diseases responsible for visual loss among the elderly population worldwide, by using gene manipulation. The aim is to introduce a gene encoding for an anti-VEGF protein inside the host cells. Thus, RGX 314 and ADVM 022 are currently under study (NCT03066258 and NCT03748784). A phase 1 trial was completed by Sanofi/Genzyme, using as a vector an AAV2 which expresses a recombinant VEGF trap, FLT01 (NCT01024998). The binding domains for both VEGF and phosphatidylinositol glycan anchor biosynthesis class F protein (PIGF), the placental growth factor of the human VEGF receptor 1, are contained by the Fms related receptor tyrosine kinase 1 (FLT01) gene. A total of 19 patients were treated by intravitreal injections and to date, both the expression of the FLT01 gene and the morphological and functional outcomes have been inconsistent (Heier et al., 2017). RGX 314, developed by REGENXBIO uses AAV8 for transporting a gene that encodes for a monoclonal antibody fragment able to neutralize VEGF. The gene is delivered specifically to retinal cells. The sustained production of an anti-VEGF protein by retinal cells would be able to reduce the number of anti-VEGF agent intravitreal injections. Another study, developed by Hemera Biosciences, is designed to assess the efficiency of HMR 59 (AAVCAGsCD59). An AAV2 vector is used, administered via intravitreal injection and the endpoint involves the inhibition of the complement cascade, by blocking the membrane attack complex (MAC). This therapy was able to reduce the proliferation of choroidal neovessels when injected into the eyes of mice in 60% of the cases and it is currently tested in treatment-naïve eyes diagnosed with AMD. MAC is involved in the formation of both choroidal neovascular membranes and geographic atrophy; thus, Hemera Biosciences is sponsoring two phase 1 studies, one for exudative AMD (NCT03585556) and one for non-exudative AMD (NCT03144999) (Cashman et al., 2011). Adverum Biotechnologies has an ongoing study on ADVM 022 genetic therapy for neovascular age-related macular degeneration. ADVM 022 utilizes AAV7m8 as a vector for genetic material encoding for aflibercept protein and can be administered via intravitreal injection. In a phase 1 study OPTIC enrolled patients that previously underwent anti-VEGF treatment, for ADVM 022 injection. At 34 weeks after receiving the treatment, ADVM 022 proved to be safe and effective, as none of the patients included needed additional anti-VEGF intravitreal injections (Grishanin et al., 2019).

In order to comparatively evaluate **micropulse vs. continuous-wave transscleral cyclophotocoagulation in neovascular glaucoma**, a total of 24 eyes from 24 patients with NVG were treated using MP-TSCPC and 22 eyes from 22 patients with NVG were treated using CW-TSCPC. The underlying causes of retinal ischemia are presented in Table 1.9. Most eyes from both groups included in the study underwent multiple

surgeries. Only 8 eyes in the MP-TSCPC group and 7 eyes in the CW-TSCPC group had no previous surgery (P=0.91). The surgery types are also listed in Table IX.

The mean follow-up period was 15.5±2.1 months (range, 12-19) for the MP-TSCPC group; all the eyes reached 12 months and 19 eyes had 15 months of follow-up. For CW-TSCPC, the mean follow-up period was 15.9±2.3 months (range, 12-21) with all the eyes reaching 12 months and 19 eyes reaching 15 months of follow-up. The percentage of favorable outcomes (or successes) at month 12 was 54.5% in the CW-TSCPC group, (95% CI, 34.1 to 73.5%), and 33.3% in the MP-TSCPC group (95% CI, 17.6 to 53.9%). The odds ratio (OR) of a favorable outcome, CW-TSCPC vs. MP-TSCPC was 2.40 [95% CI (0.73, 7.92), P=0.15]. Averaged over the 12 months of follow-up, the percentage of favorable outcomes in the two arms was 64.6% [95% CI (46.9, 79.1%] for the CW-TSCPC group, and 52.3% [95% CI (35.4, 68.7%) for the MP-TSCPC group, with an OR of 1.67 [95% CI (0.69, 4.04); P=0.25]. Results are presented in Figure 1.10 and Table 1.10.

Table 1.10 - Clinical and adverse events outcomes in the MP-TSCPC (MP) and CW-TSCPC (CW) groups

Events	CW	MP	CW vs. MP	P-value
A, Favorable outcome, proportion	% (95% CI)	% (95% CI)	OR (95% CI)	
Month 12	54.5 (34.1, 73.5)	33.3 (17.6, 53.9)	2.40 (0.73, 7.92)	0.15
Average over day 1 to month 12	64.6 (46.9, 79.1)	52.3 (35.4, 68.7)	1.67 (0.69, 4.04)	0.25
B, IOP change from baseline	Mean (95% CI), mm Hg	Mean (95% CI), mm Hg	Difference (95% CI)	P-value
Month 12	-11.95 (-17.77, -6.14)	-8.04 (-13.61, -2.48)	-3.91 (-11.96, 3.91)	0.34
Average over day 1 to month 12	-12.39 (-16.22, -8.55)	-10.04 (-13.71, -6.37)	-2.34 (-7.65, 2.96)	0.39
C, IOP (mmHg)	Mean (95% CI)	Mean (95% CI)	Difference (95% CI)	P-value
Baseline	35.82 (30.90, 40.74)	34.71 (30.00, 39.42)	1.11 (-5.70, 7.92)	0.75
Month 12	23.86 (18.07, 29.66)	26.67 (21.12, 32.21)	2.80 (-5.22, 10.82)	0.49
D, Important adverse events	Rate (95% CI)	Rate (95% CI)	Rate ratio, (95% CI)	P-value
Day 0 to month 12	0.636 (0.358, 1.030)	0.250 (0.099, 0.507)	2.55 (1.02, 7.19)[a]	0.045

[a]Rate ratio MP vs. CW. CI, confidence interval; MP-TSCPC, micropulse transscleral cyclophotocoagulation; CW-TSCPC, continuous wave-transscleral cyclophotocoagulation. IOP, intraocular pressure.

The change in IOP from baseline to 12 months was -11.95 mm Hg [95% CI (-17.77, -6.14) mm Hg] in the CW-TSCPC group, and -8.04 mm Hg [95% CI (-13.61, -2.48) mm Hg] in the MP-TSCPC group, for a difference of -3.91 mm Hg [95% CI (-11.96, 3.91) mm Hg; P=0.34]. Averaged over the 12-month follow-up, the mean change in IOP from baseline was -12.39 mm Hg [95% CI (-16.22, -8.55) mm Hg] for the CW-TSCPC group, and -10.04 mm Hg [95% CI (-13.71, -6.37) mm Hg] for the MP-TSCPC group, for a difference of -2.34 mm Hg [95% CI (-7.65, 2.96) mm Hg; P=0.39]. The mean IOP at month 12 in the two groups was 23.86 mm Hg [95% CI (18.07, 29.66) mm Hg] for the CW-TSCPC group, and 26.67 mm Hg, [95% CI (21.12, 32.21) mm Hg] for the MP-TSCPC group. Results are presented in Figure I.10 and Table I.10. The evolution of the IOP and the success rate at follow-up for both MP-TSCPC and CW-TSCPC are revealed in Table I.11.

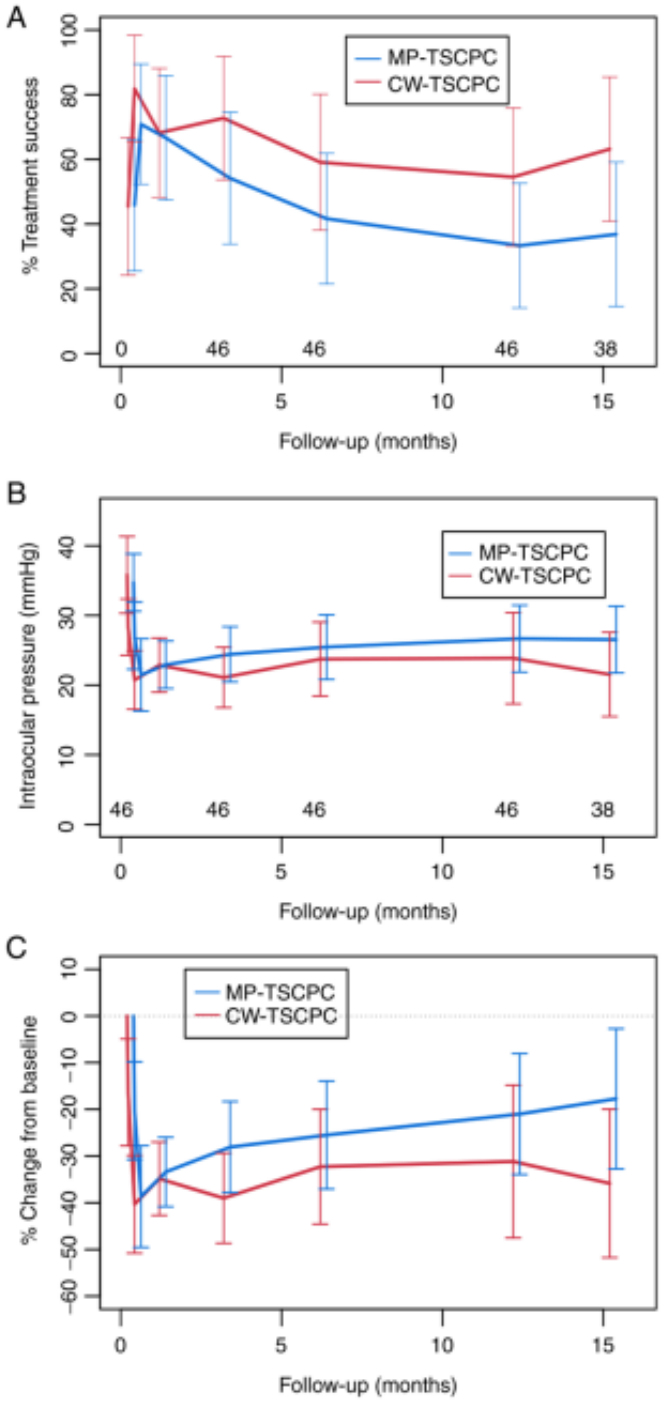


Figure 1.10 - Comparative evolution of the success rate and IOP for MP-TSCPC and CW-TSCPC.
Comparative evolution of (A) the success rate, (B) the IOP, and (C) the reduction of IOP expressed as a percentage (%) from the baseline IOP for the two methods with time-points marked at baseline, week 1, month 1, 3, 6, 12, and 15.

The mean number of important adverse events over the primary 12-month follow-up was 0.636 [95% CI (0.358, 1.030) for CW, and 0.250 [95% CI (0.099, 0.507) for the MP arm. The rate ratio was 2.55 [95% CI (1.02, 7.19)] for CW-TSCPC vs. MP-TSCPC; P=0.045. Results are presented in Table 1.10.

Table 1.11 - Evolution of the IOP and of the success rate after MP-TSCPC and CW-TSCPC at different time points.

Procedure	Outcome	Baseline	1 week	1 month	3 months	6 months	12 months
MP-TSCPC	Mean IOP (mm Hg)	34.7±10.3	21.4±12.9	23.1±8.5	24.3±9.9	25.4±1.6	26.7±12
	IOP reduction (from baseline)		38.3%	32.6%	29.9%	26.8%	23.0%
	Success rate		70.8%	66.6%	58.3%	41.6%	29.1%
CW-TSCPC	Mean IOP (mm Hg)	36.0±13.2	20.7±10	22.8±9.3	21.6±10.3	23.7±12.7	23.9±15.6
	IOP reduction (from baseline)		42.5%	36.7%	40%	34.1%	33.6%
	Success rate		77.2%	68.1%	72.2%	59.1%	54.5%

IOP, intraocular pressure; MP-TSCPC, micropulse transscleral cyclophotocoagulation; CW-TSCPC, continuous wave-transscleral cyclophotocoagulation.

For MP-TSCPC, the mean number of topical antiglaucoma medications at baseline was 2.6±1; oral acetazolamide was initially used by 14 patients (58.3%). The number of topical medications decreased during the first 3 months after treatment to 1.7±1.3 and then started to increase, reaching 2.1±1.3 at 12 months. The number of patients requiring oral acetazolamide exhibited a more pronounced decrease to only 25% at the end of the first month, 16.7% at the end of the 3rd and 6th months, and 20.8% at one year. The evolution of the antiglaucoma medication after MP-TSCPC is presented in Table 1.12 and Figure 1.11.

Table 1.12 - Variation in the number of topical antiglaucoma medications and in oral acetazolamide use at different time points after MP-TSCPC and CW-TSCPC

Procedure	Medication	Baseline	1 month	3 months	6 months	12 months
MP-TSCPC	Mean number of topical antiglaucoma medications	2.6±1	2.3±1.2	1.7±1.3	1.9±1.3	2.1±1.3
	Oral acetazolamide users (%)	58.3%	25%	16.7%	16.7%	20.8%
CW-TSCPC	Mean number of topical antiglaucoma medications	2.8±0.8	1.7±1.3	1.4±1.4	1.7±0.9	1.9±1.1
	Oral acetazolamide users (%)	63.6%	38%	27.2%	13.6%	27.2%

MP-TSCPC, micropulse transscleral cyclophotocoagulation; CW-TSCPC, continuous wave-transscleral cyclophotocoagulation.

For CW-TSCPC, the mean number of topical antiglaucoma drugs used at baseline was 2.8±0.8 and 14 patients (63.6%) used oral acetazolamide. The number of antiglaucoma drops was significantly reduced after the procedure, reaching the lowest level after 3 months (1.4±1.4), and remained quite stable during the follow-up period. The same effect, but more pronounced, was achieved in the case of oral acetazolamide users. The number of patients using oral acetazolamide was the lowest at 6 months (13.6%), with a subsequent increase at 12 months (27.2%). The results are revealed in Table 1.12 and Figure 1.11.

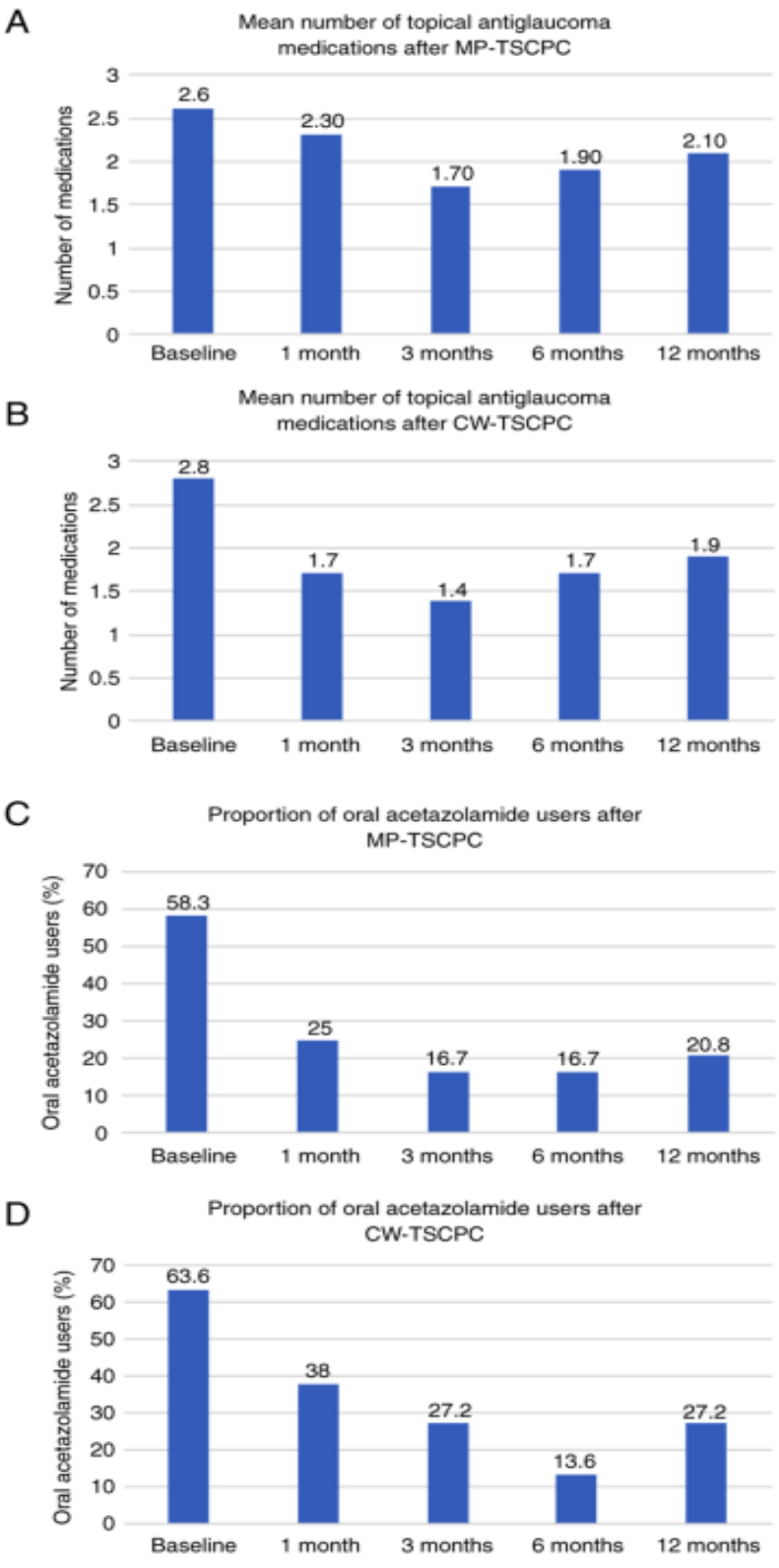


Figure 1.11 - Evolution of the number of topical medications and of oral acetazolamide used at different time points after MP-TSCPC and CW-TSCPC.
The four graphs reveal the mean number of topical antiglaucoma medications required per patient at baseline as well as months 1, 3, 6 and 12 after (A) MP-TSCPC and after (B) CW-TSCPC and the percentage of acetazolamide users at baseline and months 1, 3, 6 and 12 for (C) MP-TSCPC and (D) CW-TSCPC.

All complications encountered during our study are comparatively listed for both groups in Table 1.13. Complications were more frequent in the CW-TSCPC group. Moreover, the incidence of important adverse events (worsening of the VA, hypotony, and phthisis bulbi) was, as already stated, significantly greater in the CW-TSCPC group than in the MP-TSCPC group (P=0.045).

Table 1.13 - Complications encountered after TSCPC.

Complications	Micropulse TSCPC	Continuous wave TSCPC	P-value
Important adverse events			0.045
Worsening of visual acuity	4 (16.6%)	8 (36.4%)	0.1
Hypotony	2 (8.3%)	4 (18.2%)	0.41
Phtisis bulbi	0 (0%)	2 (9.1%)	0.22
Other adverse events			
Prolonged inflammation	1 (4.1%)	3 (13.6%)	
Choroidal detachment	1 (4.1%)	4 (18.2%)	
Postoperative intraocular pressure spike	4 (16.6%)	4 (18.2%)	
Retinal detachment	0 (0%)	3 (13.6%)	
Hyphema	3 (12.5%)	2 (9.1%)	
Neurotrophic keratitits	0 (0%)	1 (4.5%)	
Intravitreal hemorrhage	0 (0%)	3 (13.6%)	

Important adverse events include worsening of visual acuity, hypotony and phtisis bulbi. TSCPC, transscleral cyclophotocoagulation.

BCVA was at an extremely low level in our cohort at baseline. BCVA worsened in 8 (36.4) cases in the CW-TSCPC, vs. 4 (16.6%) in the MP-TSCPC group. Only the BCVA of 1 patient improved and that occurred in the MP-TSCPC group. The more frequent worsening of BCVA in the CW-TSCPC group was not statistically significant, but it reached a trend level (P=0.1), as demonstrated in Table 1.14.

Table 1.14 - Changes in VA after TSCPC.

Evolution of best-corrected VA	Micropulse TSCPC N (%)	Continuous wave TSCPC N (%)	P-value[a]
Worsened	4 (16.6%)	8-(36.4%)	0.10
Unchanged	19 (79.2%)	14-(63.6%)	
Improved	1 (4.2%)	0 (0%)	

[a]P-value from Wilcoxon rank-sum test. TSCPC, transscleral cyclophotocoagulation; VA, visual acuity.

Hypotony was present in 4 cases (18.2%) in the CW-TSCPC group and 2 cases (8.3%) in the MP-TSCPC group (P=0.41). The devastating complication of phthisis bulbi appeared in 2 cases (9.1%), both in the CW-TSCPC group. Retreatment was necessary in 6 cases in the MP-TSCPC group; 4 cases underwent retreatment 3 months after the first procedure, one case after 4 months, and one case after 6 months. In the CW-TSCPC group, 7 cases were retreated, most of them after 3 months (5 cases), one case after 6 months, and one case after 10 months.

1.4.5. Discussions

Concerning **ocular uveal melanoma**, the differential diagnosis can be difficult. For circumscribed iris melanoma, it may include iris nevi, ocular melanocytosis, different iris nodules (sarcoidosis, juvenile xanthogranuloma), iris cysts, essential iris atrophy, iris

foreign body, other iris tumors (leiomyoma), and metastasis. Diffuse iris melanoma should be mainly differentiated from diffuse iris nevi, ocular siderosis, pigmentary glaucoma, and congenital heterochromia. The most frequent posterior pseudomelanomas are represented by choroidal nevi, peripheral exudative hemorrhagic chorioretinopathy (PEHCR), congenital hypertrophy of the retinal pigment epithelium, hemorrhagic detachment of the retina or pigment epithelium (PED), choroidal detachment, circumscribed choroidal hemangioma, choroidal osteoma, and metastatic tumors (Shields et al., 2014). The main goals of ocular melanoma treatment are to destroy the tumor, prevent recurrence and metastasis, and conserve vision. While the treatment of primary uveal melanoma has constantly improved over time and different irradiation procedures have successfully replaced enucleation in selected cases, the therapeutic options for metastatic disease are still disappointing. The therapeutic attitude in primary uveal melanoma must take into account the tumor size, location and extension, the visual function, the status of the fellow eye, the age and health status of the patient, and last but not least the presence of metastasis. Usually, small uveal lesions are closely monitored clinically and with sequential photography. While UBM is a helpful adjunct in monitoring iris lesions, B-scan ultrasonography is mandatory to detect any signs of growth of posterior lesions.

Enucleation is nowadays still indicated in the treatment of large uveal melanomas (>18 mm in basal diameter and >12 mm in thickness), in cases with total visual loss due to severe complications and in tumors refractory or recurrent to conservative treatments. Even in cases with advanced orbital tumor extension, the current treatment is more conservative avoiding orbital exenteration by combining enucleation with local radiation therapy. The surgical excision of choroidal melanomas has limited indications on small tumors only.

Conservative treatment using a radioactive plaque temporarily sutured on the sclera adjacent to the tumor is one of the oldest, most effective and widespread methods for controlling medium-sized posterior uveal melanomas and has good indication especially when proton beam therapy is not available.

Unfortunately, uveal melanoma responds poorly to current chemotherapies, chemoimmunotherapy, and molecular-targeted therapy.

Regarding **gene therapy,** the majority of the current trials assess the efficacy of gene augmentation in order to obtain a functional protein as expressed by the defective gene. However, in disorders that are caused by gain-of-function genetic errors, it is necessary to identify a strategy for gene inhibition. The CRISPR/CAS9 gene-editing technique may be effective in achieving this goal. CAS9 nuclease and guide ARN can be delivered by viral vectors. Several eye diseases, including AMD or retinopathy of prematurity, are caused by polygenetic mutations. In order to develop effective gene therapy for these types of diseases, various approaches are required. One of these may involve the targeting of neurotrophic factors, either by the expression of an anti-VEGF protein or via the inhibition of the degenerative pathway. The AAVs are currently the most commonly used vectors in ophthalmic gene therapy, with AAV2 and AAV8, being the usual choice, due to their low immunogenicity and the reduced rate of side effects as compared with other possible vectors. However, their capacity for genetic data transport is only 5 kb DNA (Salganik et al., 2015). Numerous important genes that encode functional and structural proteins are larger; thus, a different vector system is required. There are studies on animal models of Usher syndrome and Stargardt disease, regarding a double-vector system, each carrying a fragment of the encoded protein, which then suffers intermolecular recombination to obtain the final product (Trapani et al., 2014).

Another study direction in the gene therapy of retinal diseases is the assessment and the enhancement of the clinical response and the measurement of the transgene expression efficiency in the target tissue (Lee et al., 2019). The evaluation of visual function is mostly achieved by measuring the visual acuity and the visual field; however, these investigations do not completely reflect the function of the entire retina. Electrophysiology testing and optical coherence tomography could improve the assessment of the morphological and functional results. A specific multi-luminance mobility test was developed for the Leber congenital amaurosis patients that receive gene therapy in order to evaluate their night blindness (Chung et al., 2018).

Concerning the study on **neovascular glaucoma (NVG),** it is important to mention that is one of the most difficult types of glaucoma to be treated. Numerous treatment methods have been used with varying degrees of success. TSCPC is a classic method used to treat NVG, belonging to the broad category of cycloablative procedures. This method has some advantages: i) it is incision-free, and thus, has a very low risk of infection; ii) it is easy to perform (in the operating room or even in an office setting); iii) it has a very short learning curve (compared with trabeculectomy and glaucoma drainage devices); iv) there is no need to stop anticoagulants; v) there is a rapid onset of the effect; and, what is more, vi)it is repeatable. The results of our study confirmed that TSCPC can be a safe and reliable method for managing NVG, using both of its variants, MP-TSCPC and CW-TSCPC, each of them with advantages and disadvantages. A successful result was defined as a postprocedural IOP between 5 and 21 mm Hg with or without additional medications or an IOP reduction of more than 30% compared with the baseline.

Hypotony, defined as an IOP of <5 mm Hg was considered to be a failure of the treatment. The definition of a successful result is a major problem because it shows great variation among different studies. The majority of other studies defined success as an IOP between 5 and 21 mm Hg (Ramli et al., 2012, Murphy et al., 2003, Iliev et Gerber, 2007); other studies also included a reduction in IOP with at least 30% from the baseline, as in a study by Aquino et al., or a reduction with at least 20% from the baseline IOP, as in a study by Grueb et al., Our cohort included patients with advanced NVG, with high initial IOP and poor initial VA and therefore it was considered that a reduction of IOP with 20% would be inefficient in most cases.

MP-TSCPC proved to be short-term effective. The success rate was 70.8% after the first week, 66.6% after the first month, and 58.3% after 3 months. The success rate decreased after three months and reached a level of only 29.1% at 12 months. Numerous studies have reported better results than ours for MP-TSCPC. Tan et al reported a success rate of 80% after 18 months of follow-up, Aquino et al revealed a 75% success rate after 12 months, Preda et al., reported a 65.63% success rate at 18 months, and Zaarour et al showed a 73.3% success rate at 12 months, but all of these studies included patients with different forms of refractory glaucoma, not only NVG. Studies on cohorts with NVG revealed slightly poorer outcomes, but some reported improved results, for example, Wong et al reported a 26% success rate at 12 months and Souissi et al revealed a 35% success rate at 9 months (Wong et al., 2020, Souissi et al., 2021). A total of 2 possible explanations were identified for this difference. Firstly, our cohort was composed of patients with advanced NVG (extremely low VA, multiple surgeries) and, secondly, the laser settings, which were not at the highest possible level of energy, may have contributed to inadequate control of IOP in the long term. It is possible to achieve satisfactory IOP control in patients with NVG, but an increase in the duration of laser delivery may be necessary. A study by Williams et al revealed improved results with a 74.7% success rate at 3 months with a longer time interval of laser application, of up to

360 sec, while using the same power. CW-TSCPC exhibited a more constant effect during the follow-up period. The success rate was 77.2% after the first week, 68.1% after the first month, 72.2% at 3 months, and 54.5% at 12 months, results that are similar to those of other studies. Singh et al., reported a success rate of 70% and Grueb et al., a 36.7% success rate, with different periods of follow-up.

Both methods resulted in a decrease in the number of topical antiglaucoma medications in the short term, which is similar to the results reported by other studies. MP-TSCPC had the most important reduction in medication number at 3 months, with the mean number of medications decreasing from a baseline of 2.6±1 to 1.7±1.3, which was followed by a slight increase to 2.1±1.3 at 12 months. CW-TSCPC had similar results at 3 months, a decrease from 2.8±0.8 at baseline to 1.4±1.4, but the results tended to be more stable in time reaching 1.9±1.1 at 12 months. The possibility to stop the carbonic anhydrase inhibitor was an important endpoint of the study and both methods proved effective in reaching this goal. The number of patients requiring oral acetazolamide decreased after both procedures: from 58.3% of patients at baseline to 20.8% at 12 months in the MP-TSCPC group and from 63.6% of patients at baseline to 27.2% at 12 months in the CW-TSCPC group. Numerous studies have reported a decrease in the number of antiglaucoma medications similar to our results (Aquino et al.,2001, Zaarour et al., 2019, Nabili et al., 2004).

Postoperative complications appeared in both groups, but the incidence was higher in the CW-TSCPC group. There were 15 complications in 9 patients (37.5%) in the MP-TSCPC group and 34 complications in 15 patients (68.2%) in the CW-TSCPC group. Potential complications in CW-TSCPC are considered to be secondary to damage induced to the surrounding tissues by the thermal effect of the laser. In MP-TSCPC the pulsatile pattern of the laser energy delivery prevents excessive heating of the collateral tissues and reduces the rate of complications (Ma et al., 2019). It is difficult to establish a cause-and-effect relationship between TSCPC and the complications, because some may appear as complications of the initial disease as, tractional retinal detachment and intravitreal hemorrhages in diabetic retinopathy, as well as late hyphema (2 cases in the MP-TSCPC group and one case in the CW-TSCPC appeared more than 3 months after the procedure). The most frequent complication was a decrease in VA; this occurred in 16.6% of cases in the MP-TSCPC group and 36.4% in the CW-TSCPC group. In other studies worsening of the VA varies from 0 to 55.2% of the cases (Ishida et al., 2013, Ocakoglu et al., 2005, Vernon et al., 2006). However, it is difficult to compare our results with those of other studies, because in our study the baseline BCVA was already extremely poor (CF or less). Herein, although a higher incidence of VA decline was reported after MP-TSCPC than in other studies such as Aquino et al who revealed a 4% deterioration of BCVA, and Elhefney et al and Lee et al who revealed no decline in BCVA, the fact that some of the cases probably had a decrease in VA as a result of the evolution of the disease, and not as a result of the procedure itself, must be taken into account. Even though the difference between the two groups was not statistically significant, it reached the trend level, and therefore MP-TSCPC is considered to be safer than CW-TSCPC in what post-procedural BCVA is concerned. Another serious complication that was identified in our study was ocular hypotony. It appeared in 2 patients (8.3% of cases) in the MP-TSCPC group and 4 patients (18.2% of cases) in the CW-TSCPC group. Unfortunately, two of these cases finally progressed to phthisis bulbi (9.1% of cases). In other studies, some reported an incidence of hypotony similar to ours in the case of CW-TSCPC, including Iliev and Gerber with 17.6%, and Walland with 18%, while some reported a higher incidence such as the study by Nabili and Kirkness, but there were also studies reporting a much lower incidence including studies by Vernon

et al and Schlote et al., Studies with a similar or higher incidence of hypotony included a greater percentage of patients with NVG, whereas the other ones had few or no patients with NVG. This may be explained by the fact that eyes with NVG have a disproportionate outflow resistance, while the aqueous humor production is already damaged by ischemia. As such, any cyclodestructive procedure, even a mild one, as is the case with MP-TSCPC, can disturb the balance between outflow resistance and the aqueous production, resulting in hypotony (Nabili et al., 2004). Our study revealed that, concerning adverse effects (i.e., the decrease in VA, hypotony, and phthisis bulbi), their higher occurrence rate in the CW-TSCPC group vs. in the MP-TSCPC group was statistically significant (P=0.045).

The most important limitation of our study resides in the small sample size for each group (24 and 22 cases, respectively, for MP-TSCPC and CW-TSCPC). Another limitation emerges from the retrospective nature of the study and from the fact that not all the data were available for all the patients. However, the patients were observed concurrently by the authors and followed the same visit schedule, what is more, the study had a 12-month follow-up on 46 of the 51, or 90%, of the patients who underwent the intervention. Our center is a tertiary care center, and thus the postoperative visits of some of the patients took place in their primary care center. A lot of effort was made to retrieve the data from those primary care centers, however, it cannot be certain that the accuracy of the data is the same.

1.4.6. Conclusions

The reviews on **uveal melanoma** and **gene therapy for retinal diseases** have revealed interesting conclusions. The prognosis, survival rate, and quality of life in primary uveal melanoma tumors have significantly improved since the introduction of conservative irradiation therapies and surgical excisions. On the contrary, despite the consistent knowledge that has been acquired in the last decades regarding tumor genetics and pathogenesis (especially the biological and immunological mechanisms leading to tumor growth and spreading), there is to date no efficient therapeutic algorithm for controlling the metastatic disease responsible for a quick fatal outcome in almost 50% of the patients. Hopefully, the multiple clinical studies ongoing on this topic will soon confirm the encouraging preliminary results leading to more efficient and safer therapeutic protocols that will consistently increase the survival rate of these patients. In the field of gene therapy for retinal diseases, despite significant advances, many unanswered questions remain. These are regarding the safety profile of the promoters, the unanticipated side-effects of gene manipulation that may develop in the long-term, as well as the economical implication of this type of complex therapy. Nonetheless, the emerging field of gene therapy has yielded promising results in finding cures for several debilitating and blinding diseases, which were considered intractable to date.

In terms of NVG treatment, our clinical study has demonstrated that both laser methods, **MP-TSCPC and CW-TSCPC**, can successfully manage this particular type of glaucoma. While CW-TSCPC exhibited higher IOP control in the long term (not statistically significant), it showed a significantly lower safety profile. On the contrary, MP-TSCPC has proved to be safer, but its efficacy seems to decline after three months. Also, patients with advanced NVG required higher laser energy or longer application time to obtain the required result.

1.5. Modern vitreoretinal surgery: controversies and a critical evaluation of different techniques and adjuncts

1.5.1. Introduction

Robert Machemer, MD (1933-2009) is considered the "Father of Vitreous Surgery" for the introduction of pars plana vitrectomy (PPV) in 1972. This new technique revolutionized the outcome of many invalidating diseases, like diabetic retinopathy, retinal detachment, and vitreous opacities in an era when such conditions posed a great challenge for ophthalmologists.

As expected, the technique of pars plana vitrectomy quickly evolved from a single-port instrument with combined infusion, suction, and cutting abilities, that entered the vitreous cavity through a 2.3-mm sclerotomy to a 3-port 20-G system, firstly developed by Malley and Heintz in 1975. The external source of illumination was drastically improved and integrated as intraocular fiberoptic illumination. The outstanding results reported with this technique encouraged the retinal surgery field and the significant enlargement of vitrectomy indications.

During the years, the classical 20-G PPV (0.91 mm diameter), performed through a 1.15-mm incision, evolved into a 25-G instrument system (introduced by Fujii et al., in 2002), with a 0.51 mm diameter vitrector, and soon after that to the 23-G system in 2005 (created by Eckardt), that combined the benefits of the both 20- and 25-G systems. Thus, due to the sutureless closure, the era of modern microincision vitrectomy surgery (MIVS) began. Moreover, Oshima and colleagues introduced in 2010 the 27-G instrumentation, with a vitrector with an outer diameter of only 0.409 mm and an inner diameter of 0.275 mm. Despite its smaller diameter, and also that the 27-G system demonstrated equivalent to the 25-G system at cut rates between 1000 and 1500 cpm, it is mainly used in limited procedures due to the instrumentation limitations and delicacy. Nowadays, the most common procedures are performed with 23G or 25G systems.

The MIVS systems are less traumatic to conjunctival and scleral tissues, avoid irritating sutures, contribute to reduced inflammation and scarring and promote faster recovery and improved postoperative comfort. Most platforms provide nowadays facilities for both anterior and posterior segment surgery, thus combined surgery is today achievable.

This direction of research is reflected in the following published article:

Moraru A D, Costin D, Moraru RL, **Branisteanu DC.** Outcomes of simultaneous vs. sequential pars plana vitrectomy and cataract surgery. Experimental and Therapeutic Medicine, 2020, 20(6): art. no 183. **IF=1.785**

https://doi.org/10.3892/etm.2020.9313

The risk of iatrogenic retinal tears is also significantly reduced. Reducing the instrumentation diameter has obvious advantages. Still, the greater flexibility of such small-gauge instruments reduces the surgeon’s access to the periphery of the retina, as the instruments are less efficient in rotation of the globe. Due to the improved efficacy and safety of the technique and also due to the variety of instrumentation the pars plana vitrectomy has enlarged the classical spectrum of indications toward a multitude of pathologies that include retinal detachment, macular hole, macular hemorrhages, vitreomacular traction, epiretinal membrane, vitreous hemorrhage, vitreous biopsy,

intraocular foreign body, endophthalmitis, intraocular lens (IOL) exchange, and removal of the dropped lens nucleus.

This direction of research is reflected in the following published article:

Ochinciuc R, Balta F, **Branisteanu DC**, Burcea M, Zemba M, Ochinciuc U, Barac R. Subretinal alteplase injections in massive subretinal hemorrhage due to age-related macular degeneration: A case report series, Experimental and Therapeutic Medicine, 2020, 20(6): art. no 208. **IF=1.785**

https://doi.org/10.3892/etm.2020.9338

The outstanding results obtained today with pars plana vitrectomy in various pathologies are highly dependent on the concomitant usage of various adjuncts such as the intraocular dyes, heavy liquids, gases, and silicone oil (SIO). Among them, silicone oil is of particular interest, as it is, up to this moment, the only adjunct that can provide long-term intraocular tamponade so necessary to allow stability. The most important differences among silicone oils are related to the molecular weight (MW), the length of the linear chain, the chemical structure of radical side groups, the termination of the polymer chains, and the size distribution of the chain. The viscosity of different types of silicone oil is expressed in centistokes (1 cs = 10−6 m2/s). Enhancing silicone oil's molecular weight will increase its viscosity. Silicone oils currently used in vitreoretinal surgery have a viscosity ranging from 1.000 (MW 37 kDa) to 5.000 cs (MW 65 kDa). Due to their tendency to emulsify and generate complications SIO must be removed, usually after several months. The main issues regarding the use of silicone oils are the rate of pathology reoccurrence after the silicone oil removal and also about the intraocular pressure changes during and after SIO endotamponade.

This direction of research is reflected in the following published articles:

Branisteanu DC, Moraru AD, Maranduca MA, Branisteanu DE, Stoleriu G, Branisteanu CI, Balta F. Intraocular pressure changes during and after silicone oil endotamponade (Review). Experimental and Therapeutic Medicine, 2020, 20(6): art. no 204. **IF=1.785**

https://doi.org/10.3892/etm.2020.9334

Branisteanu DC, Moraru A, Bilha A. Anatomical results and complications after silicone oil removal, Romanian Journal of Ophthalmology, 2017, 61(4): 261-266 (Pubmed)

As a vitreoretinal surgeon, I have spent most of the time in the operating room approaching, besides primary retinal detachments, different complications related to the diabetic retinopathy, various macular pathologies, intraocular foreign bodies, or posterior luxated lens fragments. Some of the indications for vitrectomy, such as persistent neovascularization and refractory macular edema, have faded in time as intravitreal therapy with anti-VEGF agents and steroids proved to be more efficient, easier, and safer.

I have personally contributed to the implementation of classical 20G vitrectomy in Romania in the early 2000's, and after that, made the conversion to modern microincision vitrectomy surgery. Thus, I had the occasion to largely evaluate the efficacy of vitrectomy in various hot topics like luxated lens fragments, various macular diseases, persistent macular edema, or rare conditions like ocular toxocariasis, Terson's syndrome, or acute

retinal necrosis. Moreover, I could personally compare the differences between modern smallG vitrectomy and 20G surgical procedures regarding efficacy, safety, and postoperative rehabilitation.

I have tried to help elucidate some of the controversies at that time regarding different surgical techniques suggested in diabetic retinopathy complications surgery, such as the efficacy of air tamponade in the prevention of early recurrence of vitreal hemorrhage or to compare surgical versus laser therapy approach in persistent diabetic macular edema.

This direction of research is reflected in the following published articles:

Branisteanu DC, Bilha A, Moraru A. Vitrectomy surgery of diabetic retinopathy complications, Romanian Journal of Ophthalmology, 2016, 60(1): 31-36 (Pubmed)

Moraru A, Mihailovici R, Costin D, **Branisteanu D.** Terson's syndrome. case report, Romanian Journal of Ophthalmology, 2017, 61(1): 44-48 (Pubmed)

Branisteanu D, Moraru A. Therapeutic approach in persistent diabetic macular edema, Oftalmologia, 2014, 58(4): 3-9 (Pubmed)

Branisteanu D, Moraru A. Macular surgery in a new point of view, Oftalmologia, 2014, 58(3): 49-54 (Pubmed)

Moraru A, Panfil M, Totolici G, **Branisteanu D**, Costin D, Schmitzer S. Ocular toxocariasis - case report, Oftalmologia, 2014, 58(4): 30-35 (Pubmed)

Branisteanu D, Moraru A. Internal limiting membrane role in primary surgery of the macular hole, Oftalmologia, 2013, 57(4): 44-50 (Pubmed)

Branisteanu D, Moraru A. Surgery for posterior segment intraocular foreign bodies - anatomical and functional results, Oftalmologia, 2013, 57(4): 51-60 (Pubmed)

Branisteanu D, Moraru A. Surgery in posterior luxated lens fragments through intravitreal phacofragmentation, Oftalmologia, 2013, 57(3): 66-72 (Pubmed)

Branisteanu D, Robu M, Antohi I, Medvichi R. Acute retinal necrosis syndrome, Oftalmologia, 2007, 51(4): 57-64 (Pubmed)

Branisteanu D, Robu M, Irimia A. Persistent diabetic macular edema - surgical versus laser therapy, Oftalmologia, 2007, 51(1): 74-79 (Pubmed)

Branisteanu D, Danielescu C, Irimia A, Robu M. Efficiency of air tamponade in prevention of early recurrence of vitreal hemorrhage in diabetic patients with vitrectomy, Oftalmologia, 2006, 50(2): 62-67 (Scopus)

1.5.2. Aim

The coexistence of cataracts with vitreoretinal diseases is common in clinical practice, not only in elderly patients but also after eye trauma, uveitis, and diabetes. The presence of both disorders in the same eye renders the case to be surgically challenging and the evolution to be somewhat unexpected. A dense cataract in an eye with vitreoretinal pathology requiring surgical intervention can make routine surgery difficult and raise the intra and postoperative rate of adverse reactions. On the other hand, it is a known fact that cataract progresses after posterior eye segment surgery. As both medical and economic factors influence the decision of the surgeon in choosing the appropriate procedure for the patient, the current trend in Europe is to perform a combined procedure for cases presenting with both pathologies, as opposed to US surgeons. Presumably, the combined procedure is prone to an increased rate of postoperative complications such as intraocular inflammation and an increase in intraocular pressure. Nevertheless, when sequential surgery is chosen and phacoemulsification is performed after pars plana vitrectomy (PPV), the cataract extraction intervention is more difficult due to either a harder nucleus or due to different morphological conditions in a vitrectomized or silicone oil-filled eye. The present study aimed to comparatively assess the efficacy (through the morphofunctional results) and safety of simultaneous, **combined phacoemulsification and vitrectomy vs sequential surgery**, in a series of patients presenting both pathologies.

Massive subretinal hemorrhage (SRH) is a devastating complication that can occur in patients with both wet and dry age-related macular degeneration (AMD). Due to the toxic effect of the accumulated iron compounds on the photoreceptors and retinal pigment epithelium (RPE), the therapeutic solution must be found very quickly, otherwise, the visual prognosis is reserved. Subretinal injections with recombinant tissue plasminogen activator (rTPA) have been used for some time and the results are highly variable from case to case. **Alteplase** is a thrombolytic agent, a glycoprotein, produced by recombinant deoxyribonucleic acid (DNA) synthesis in cell culture (Michel et al., 2019). It is approved by Food and Drug Administration for intravenous administration in acute ischemic stroke, pulmonary embolism, acute myocardial infarction, and occluded catheters (Harvison, 2007). From its inactive form, it becomes active after fibrin coupling, which eventually results in the transformation of plasminogen into plasmin. Without treatment, in a massive SRH, in the most common cases, the final visual acuity of the patients is only light perception. The subretinal use of rTPA led to the improvement of these results (Fine et al., 2017, Singh et al., 2006), despite possible retinal toxicity observed in cats and rabbits (Johnson et al., 1990, Hrach et al., 2000). Moreover, using immunostaining with antibodies against brain-specific home box/POU domain protein 3a (Brn3a), in a mouse model of glaucoma, degeneration of retinal ganglion cells was observed, determined by up-regulating of the tissue plasminogen activation (Chintala, 2016). Brn3a is a transcription factor expressed in the central and peripheral sensory nervous system such as trigeminal ganglion or retinal ganglion cells (RGCs) in rodents and probably in humans. It was shown that the increase in IOP caused the increase of the proteolytically active tPA, which led to a reduction of Brn3a and RGCs in mice. However, there is no consensus regarding the indications for this procedure, the dose of the substance, not even the surgical technique. In order to evaluate the efficacy and safety of this technique, an interventional study on consecutive cases of massive SRH related to AMD was performed.

Silicone oil (SIO) and perfluorocarbon liquids (PFCLs) are one of the most important adjuncts used in vitreoretinal surgery. Due to their systematic usage during pars plana vitrectomy, the majority of complicated forms of retinal detachments can be fixed and stabilized. While PFCL usage is restricted only intraoperatively, SIO provides the desired long-term postoperative endotamponade and has expanding indications such as ocular trauma, advanced diabetic retinopathy, viral retinitis, macular holes, or chronic uveitis. Also, SIO is the preferred option in patients unable to maintain positioning or have to travel by air in the early postoperative period. Maintaining significant adhesion of neurosensory retina to the retinal pigment epithelium is the direct consequence of surface tension, SIO being immiscible with water. Increased viscosity provides more surface tension and also less emulsification rate as indicated by in vitro studies. In real life, these advantages might not be clinically significant if endotamponade lasts no more than 6 months. Despite their highest degree of purity emulsification can still occur, unpredictably and irrespective of SIO viscosity (1000 or 5000 cs). Longer direct contact of SIO with PFCL during vitrectomy or with different biological emulsifiers inside the eye during endotamponade (blood, proteins, or inflammatory mediators) has been associated with an increased risk of emulsification (Joussen et Wong, 2008, Barca et al., 2014). Although lower viscosity SIO is easier to be removed during modern Micro-Incisional Vitreoretinal Surgery (MIVS), many surgeons still prefer to use higher viscosity SIO due to increased surface tension and lower emulsification rate, especially when longer endotamponade is anticipated. To achieve efficient endotamponade to the inferior retina, a particular heavier than water SIO was created (Densiron 68 and Oxane HD). In this review, we will restrict the discussion to the most commonly used SIO, which is lighter than water and currently has a viscosity between 1,000 (MW 37 kDa) and 5,000 cs (MW 65 kDa).

Although the literature does not indicate a precise timing for extraction, SIO is usually removed after 3 to 6 months postoperatively, to prevent complications especially related to emulsification. Of course, the anatomical result is the key factor influencing the surgeon's judgment regarding the best timing.

Many complications have been associated with SIO endotamponade. While refractive change is a perturbing yet harmless feature during endotamponade, more severe complications may occur as macular pucker, cystoid macular edema, and rubeosis iridis. Long-standing SIO endotamponade is associated with significant ocular complications such as cataract formation, corneal decompensation, band keratopathy, and orbital leakage (Morphis et al., 2012, Lemaitre et al., 2020). Silicone oil neuropathy is the consequence of the direct toxic effect on the optic nerve. Retrolaminar, chiasmal, and even brain migration of SIO vacuoles have been documented (Poillon et al., 2019).

Among all complications related to SIO endotamponade, intraocular pressure (IOP) changes induced by tamponade itself or by SIO extraction are one of the most common and one published review aims to update the most recent information on this topic. Instead, the most important complication after SIO removal is retinal detachment recurrence. In literature, the incidence largely varies between 3.5 and 34%. Patient selection, surgical techniques, and time before SIO removal are considered significant factors influencing the result (Al-Wadani et al., 2014). Primary disease is also very important and influences the rate of reported recurrence of retinal detachment after SIO removal. The rate seems to be higher in eyes with severe PVR and complicated trauma and lower in proliferative diabetic retinopathy and giant retinal tears (Morphis, 2012). The purpose of another published study was to evaluate the anatomical results after silicone oil removal in a personal series of cases, as well as the complications, encountered intra and postoperatively.

Diabetic retinopathy is one of the leading causes of visual loss in both the elderly and the working-age population. Danaei et al., reported in "The Lancet" that age-standardized adult diabetes prevalence has reached 9.8% in men and 9.2% in women. Approximately 24% of these patients are already diagnosed with different forms of diabetic retinopathy but 28% will remain undiagnosed until the onset of complications. The prevalence of diabetic retinopathy grows proportionally to the duration of diabetes, so all the patients with type 1 diabetes and 60% of those with type 2 diabetes will be diagnosed with a form of diabetic retinopathy after 20 years of disease.

The diabetic retinopathy affects the retinal microvascularization, leading to progressive retinal ischemia, neovascularization, and fibro-cellular proliferation. Many patients are referred to a retina specialist in the late phases of diabetic retinopathy evolution when severe complications like vitreous hemorrhage and tractional retinal detachment are already installed. On the other hand, 5% of the patients with diabetic retinopathy, appropriate ophthalmic care, and strict metabolic control still develop ocular complications requiring surgical treatment.

The first pars plana vitrectomy was successfully performed in 1970, on a diabetic eye with persistent vitreous hemorrhage, by Robert Machemer, and led to a significant increase in the anatomical and functional prognosis in these cases. This outstanding evolution toward ophthalmic microsurgery led to surgical instruments' miniaturization and the refinement of surgical techniques. Today, minimally invasive small G transconjunctival pars plana vitrectomy (with either 23G, 25G, or 27G) is the standard of care in such cases. All this time, non-clearing vitreous hemorrhage remained one of the main indications of vitrectomy in the diabetic eye. Today, the advances in surgical techniques allowed the improvement of most complex cases of retinal detachments. The other indications for surgery, such as persistent neovascularization and refractory macular edema have faded in time as intravitreal therapy with anti-VEGF agents and steroids proved to be more efficient, easier, and safer. The purpose of one publication was to assess the **anatomical and functional results after vitreoretinal surgery** in a large series of patients operated for complications due to diabetic retinopathy and to compare the 23G versus 20G surgical procedure regarding efficacy, facility, safety, and postoperative rehabilitation.

1.5.3. Material and methods

For the comparative study regarding **simultaneous vs. sequential pars plana vitrectomy and cataract surgery** a series of 87 cases, presenting with cataract and vitreoretinal pathology were retrospectively reviewed from their clinical records from 2017 to 2019. The study was approved by the Ethics Committee of 'N. Oblu' Clinical Hospital (Iași, Romania) and informed consent was obtained from each patient. The patients included in the study were diagnosed with the following vitreoretinal disorders: Rhegmatogenous retinal detachment, vitreous hemorrhage from proliferative diabetic retinopathy or blunt trauma, tractional retinal detachment all associated with cataract. Cases presenting with penetrating trauma were excluded from the study. The patients were divided into two groups: group 1 comprising 41 cases that underwent the combined procedure and group 2 comprising 46 patients that underwent PPV for the vitreoretinal pathology, followed by cataract surgery after a period of time between 3 and 10 months.

The visual acuity (VA), IOP, the anatomical outcome and the complications were assessed preoperative and postoperative. The VA was tested on the Snellen chart. The IOP was measured by Goldmann tonometry. Both the anterior and the posterior segment of the patients' eyes were examined at the slit lamp before surgery and in the first and

third day postoperative. After discharge, the cases returned for follow-up at 2 weeks, and at 1, 3, and 6 months. The intraocular lens (IOL) power was assessed by optical biometry, or in the case of dense cataracts by ultrasound biometry, using the SRK-T formula. An ultrasound of the posterior segment of the eye was performed in cases of dense cataracts.

The surgical procedure consisted of phacoemulsification of the lens by clear corneal incisions and 23 or 25 G PPV, under peribulbar anesthesia, after obtaining the informed consent of the patient. In cases that underwent the combined procedure, the trocars were put in place before the cataract extraction. The phacoemulsification was performed using one 2.2 mm main incision and two 1.2 mm side ports, under the protection of one cohesive and one dispersive ophthalmic viscosurgical device (OVD). A hydrophobic, foldable, acrylic IOL was placed in the capsular bag or ciliary sulcus at the end of the cataract surgery. If phacoemulsification and PPV were performed simultaneously, the main corneal incision was secured by a 10.0 nylon suture during vitrectomy. In all cases requiring endotamponade, 1000 cS or 5000 cS silicone oil was used.

In the postoperative period, the patients have prescribed a topical antibiotic and steroidal anti-inflammatory drugs 5 times a day for 1 week and tapered until the end of the first month. A few cases needed cycloplegic therapy with topical mydriatics 2-3 times a day for 1 week.

The statistical analysis was done using MaxStat software and the data were expressed as either percentage or mean values. $P<0.05$ was considered statistically significant.

In order to evaluate **the efficacy and safety of subretinal alteplase injections in massive subretinal hemorrhage (SRH) due to age-related macular degeneration,** an interventional study was performed accordingly to the Institutional Guidelines of the Ponderas Academic Hospital, Bucharest, Romania. Approval was obtained from the Institutional Review Board and the Ethics Committee of the Ponderas Academic Hospital. All procedures conformed to the tenets of the World Medical Association Declaration of Helsinki. All patients gave their written informed consent.

Three cases of SRH were analyzed. In all cases, a 25-gauge pars plana vitrectomy (PPV) with hyaloid removal was performed. In eyes with vitreous hemorrhage, all blood and membranes were removed from the retinal surface. For the subretinal injection, a 1 ml insulin syringe and a 38-gauge cannula were used. Actilyse® 50 mg (Boehringer Ingelheim International GMBH, Germany) was used. After the powder and solvent were properly mixed according to the instructions, 0.6-0.7 ml of solution was drawn into the syringe. On average 0.3 ml of the substance was injected through the lower part of the macula until a bullous retinal detachment was obtained. Depending on the size of the clot, more or less of the substance was injected or a second injection was made in another part of the macula. In some cases, a quick dislocation of the clot through the injected site was observed. After total fluid-air exchange, 1.25 mg/0.05 ml bevacizumab (Avastin®, Roche, Switzerland) injection was intravitreally injected in all patients (12). A Face-down position was recommended in all cases for the next 24 h. Patients were evaluated on the first day, 1 week, and 1 month after surgery.

For the review on **intraocular pressure changes during and after silicone oil endotamponade,** the authors performed an extensive literature search in the Medline electronic database, using the PubMed interface. The keyword combinations used were ‘intraocular pressure’, ‘silicone oil’, and, in turn, each of the following: ‘changes’, ‘treatment’, “follow-up”. We included articles in English, published in the last 10 years.

After filters were applied (case report, classical article, guideline, journal article, meta-analysis, observational study, review, systematic review) a consistent number of references resulted. Among these, 44 references were cited in this review. Also, personal experience in the field is mentioned in the review, as a result of a retrospective evaluation of a significant number of cases that underwent SIO endotamponade.

The anatomical results and complications after silicone oil removal were evaluated during a retrospective, interventional study, evaluating consecutive cases that underwent SIO removal after primary 23G vitrectomy for complex retinal detachments. All cases were operated on using local anesthesia. Alcon CONSTELLATION® Vision System was used at both primary vitrectomy and later on to actively remove the silicone oil. Oxane 5700 silicone oil was used for endotamponade in all cases. The anatomical result was the main followed parameter. Intra and postoperative complications and also intraocular pressure changes were evaluated. Cases were followed up for at least 12 months.

The study on the **anatomical and functional results after vitreoretinal surgery of diabetic retinopathy complications** was interventional, retrospective, and comparative. The patients were included if one of the following complications due to diabetic retinopathy was present: non-clearing vitreous hemorrhage, vitreomacular traction syndrome (epiretinal membranes, retinal detachments, and macular heterotopia), persistent neovascularization with rubeosis iridis, persistent or tractional macular edema.

All the patients were operated on between January 2000 and December 2014. Between January 2000 and October 2011, the standard 20G vitrectomy was performed by using the Accurus/ Alcon equipment at the Ophthalmology Department in "St. Spiridon" Hospital, Iasi. Between November 2011 and December 2014, the procedure was performed exclusively on an ambulatory basis, by using 23G vitrectomy provided by Constellation/ Alcon unit in "Retina Center" private practice, Iasi. All the cases were operated on under local anesthesia. The sub-Tenon's anesthesia was mainly used during the 20G vitrectomy, and peribulbar anesthesia was performed to complete the 23G vitrectomy. The anticoagulants and antiplatelet medication were stopped, reduced, or temporarily replaced in the perioperative period.

Cases were clinically followed-up for at least 12 months. At each visit, a complete ophthalmic evaluation was performed by including the best-corrected visual acuity (BCVA), intraocular pressure and according to each case, ultrasonography, or spectral-domain optical coherence tomography (SD-OCT).

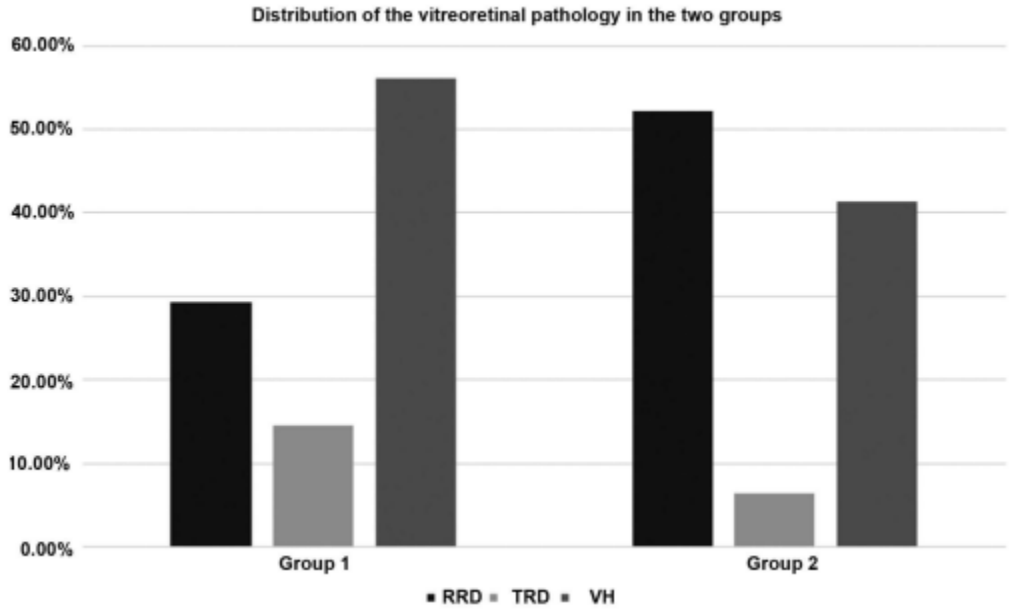
The complexity of the surgery varied largely according to the severity of each case and included complete gestures such as vitreous removal, membrane peeling (with or without dye enhancement) segmentation and/or delamination of neovascular pegs, endodiathermy, endolaserphotocoagulation, subretinal fluid removal and air, gas or silicone oil endotamponade.

1.5.4. Results

In the study of **simultaneous vs. sequential pars plana vitrectomy and cataract surgery,** eighty-seven eyes from 87 patients were included. Group 1, which underwent combined procedures was comprised of 27 (65.85%) women and 14 (34.15%) men, and group 2, in which the surgical procedures were performed separately, consisted of 28 (60.87%) women and 18 (39.13%) men. There was no significant difference between the

mean age of the groups: 58±9.42 in group 1 and 56±11.3 in group 2. The percentage distribution of the vitreoretinal pathology in the two groups is presented in Table 1.15.

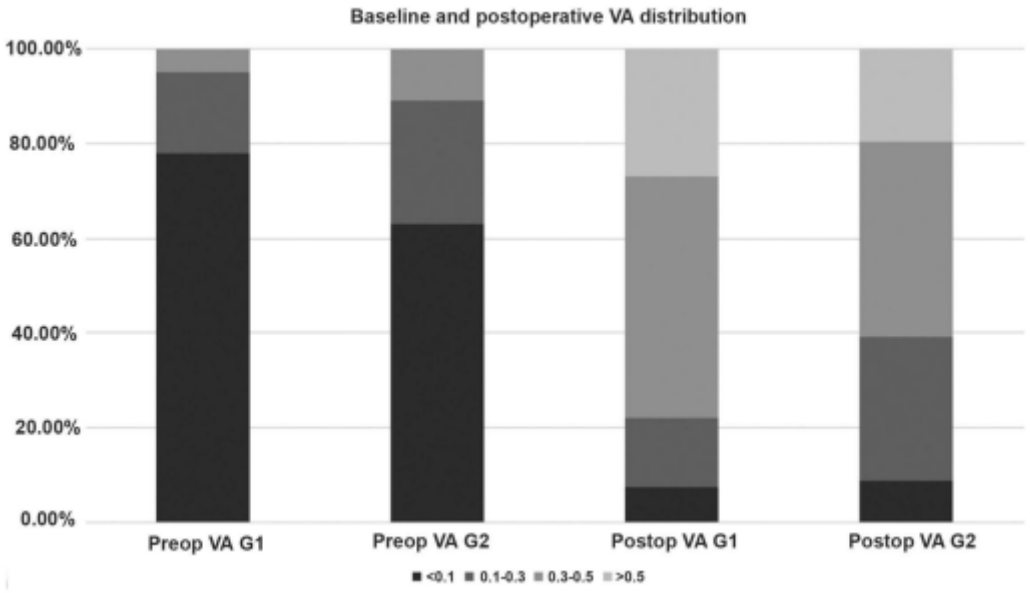
Table 1.15 - Percentage distribution of the vitreoretinal pathology in the two groups. (RRD, rhegmatogenous retinal detachment; TRD, tractional retinal detachment; VH, vitreous hemorrhage).



In group 1 there were 12 (29.27%) patients diagnosed with rhegmatogenous retinal detachment, 6 (14.64%) with tractional retinal detachment and 23 (56.09%) with vitreous hemorrhage. In group 2, 24 (52.17%) patients were diagnosed with rhegmatogenous retinal detachment, 3 (6.53%) with tractional retinal detachment and 19 (41.3%) with vitreous hemorrhage.

The preoperative best-corrected visual acuity (BCVA) did not differ significantly between the two groups, 32 (78.05%) patients from group 1 had a VA <0.1; 7 (17.07%) had a VA between 0.1 and 0.2 and 2 (4.88%) had a VA of 0.3. In group 2, a VA <0.1 was recorded in 29 (63.05%) patients, VA between 0.1 and 0.2 in 12 (26.09%) patients and a VA between 0.3 and 0.4 in 5 (10.86%) patients. The final BCVA, after surgery, in group 1 was <0.1 in 3 (7.32%) patients, between 0.1 and 0.3 in 6 (14.63%) patients, between 0.3 and 0.5 in 21 (51.22%) patients and >0.5 in 11 (26.83%) patients. In group 2, the final BCVA, after both surgeries were completed was <0.1 in 4 (8.7%) patients, between 0.1 and 0.3 in 14 (30.43%) patients, between 0.3 and 0.5 in 19 (41.3%) patients and >0.5 in 9 (19.57%) patients. The final BCVA did not differ significantly between the two groups, but there was a larger percentage of eyes with a postoperative VA comprised between 0.1 and 0.3, in group 2 (30.43%), comparative to group 1 (14.63%) and more eyes in group 1 (51.22%) had a VA of 0.3-0.5 by comparison to 41.3% in group 2. The baseline and postoperative VA distribution in both groups is presented in Table 1.16. The simultaneous surgery group had a higher rate of eyes that benefited from a final BCVA of >0.3 (78.05%), by comparison to the sequential surgery group, in which 60.87% obtained a postoperative VA of >0.3.

Table 1.16 - Baseline and postoperative VA distribution in both groups.
(VA = visual acuity).

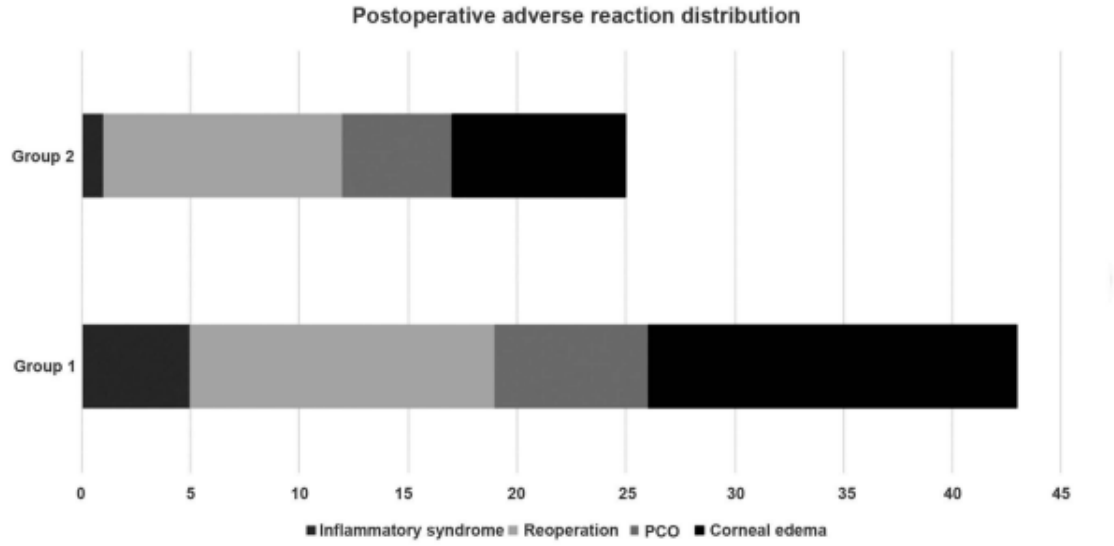


Similar values of the IOP were registered in both groups preoperative. In group 1 the mean IOP was 14±6.3 mmHg and in group 2 the mean IOP was 13±7.1 mmHg. At the end of the first month of postoperative follow-up, the mean IOP in group 1 was 18±6.4 mmHg and in group 2 was 15±8.5 mmHg. However, at the end of the first year of follow-up, the mean IOP had similar values in both groups: 13±5.1 mmHg in group 1 and 14±6.8 mmHg in group 2. One patient from group 1 (2.4%) and 3 (6.5%) patients from group 2 required topical hypotensive treatment during the whole follow-up period.

During the cataract surgery, the most common intraoperative complication was miosis present in 9 (21.95%) eyes from group 1 and 12 eyes (26.08%) from group 2, followed by posterior capsule rupture in 3 eyes (7.31%) from group 1 and 7 eyes (15.21%) in group 2, 1 (2.4%) hemorrhage in the anterior chamber (AC) in group 1 and 4 eyes (8.7%) with diminished AC depth from group 2, due to pressure exerted by the silicone oil on the posterior capsule. During PPV the most common intraoperative complication encountered was the difficulty of shaving the vitreous base in 24 eyes (52.17%) with un-operated cataract from group 2 and the difficulty of visualization of the vitreous base in 13 eyes (31.7%) with IOL from group 1. There were no iatrogenic tears registered in either group.

During the follow-up period, 15 eyes (32.6%) from group 2 registered a significant advance in cataract density and had to be operated in the third month after PPV. The rest of the eyes from group 2 benefited from cataract surgery after a period of 3-6 months after PPV - 18 eyes (39.13%) and after 6 months - 13 eyes (28.27%). On the first postoperative day, 5 (12.2%) eyes from group 1 had a mild fibrin reaction in the AC by comparison to 1 (2.2%) in group 2 after PPV and 1 (2.2%) after cataract surgery, which resolved with topical and subconjunctival injections of dexamethasone. There were no patients, in either group, with posterior synechia. Corneal edema was present in 17 (41.46%) patients in group 1, 1 (2.2%) patient from group 2 after PPV, and 7 (15.2%) patients from the same group after cataract surgery. In all cases, the corneal edema resolved during the first week postoperative with topical treatment. The postoperative adverse reaction distribution is presented in Table 1.17.

Table 1.17 - Postoperative adverse reaction distribution: Number of patients in each group presenting with a postoperative inflammatory syndrome, posterior capsular opacification (PCO), corneal edema, or in the need of surgical reintervention.



The reoperation rate of all causes was 34.14% (14 eyes) in group 1 and 23.9% (11 eyes) in group 2. Reoperation was due to: retinal redetachment in 9 (21.95%) eyes from group 1 and in 8 (17.4%) eyes from group 2; a new vitreous hemorrhage in 1 (2.4%) patient from group 1 and 3 (6.5%) from group 2 and displacement of the IOL in 4 (9.75%) cases from group 1.

The rate of posterior capsular opacification (PCO) encountered during the follow-up period was 17.07% (7 eyes) in group 1 and 10.87% (5 eyes) in group 2. Postoperative hypotony was registered in 6 eyes (14.63%) from group 1 and 2 eyes (4.34%) from group 2, after PPV, which resolved during the first week after surgery.

In the study evaluating the **efficacy and safety of subretinal alteplase injections in massive subretinal hemorrhage due to age-related macular degeneration,** three cases were analyzed.

The first case is a 74-year-old male with neovascular AMD in both eyes, with anti-vascular endothelial growth factor (VEGF) therapy in the right one, complaining of a sudden decrease of visual acuity and central scotoma in the last 2-3 days in his right eye. Due to the cardiovascular risk, the patient was treated with anti-aggregating agents and oral anticoagulants for several years. Best-corrected visual acuity (BCVA) at the presentation was hand motion. At the fundoscopic examination, a massive SRH was observed affecting the entire macular surface. Optical coherence tomography (OCT) revealed a significant retinal thickening. The patient was submitted to PPV, with subretinal rTPA injection and intravitreal bevacizumab. Fluid-gas exchange was performed at the end of the surgery. Due to gas and diffuse vitreous hemorrhage, weakly transparent, BCVA was hand motion the first 2 weeks postoperatively. One month after the surgery BCVA was 0.8 (decimal fraction), with a significant improvement of the fundoscopic and spectral-domain optical coherence tomography (SD-OCT) examination (Figure 1.12). Clinical examination revealed the presence of the blood clot inferior, below the peripheral retina. The patient followed regular checkups, with intravitreal injections of bevacizumab as needed.

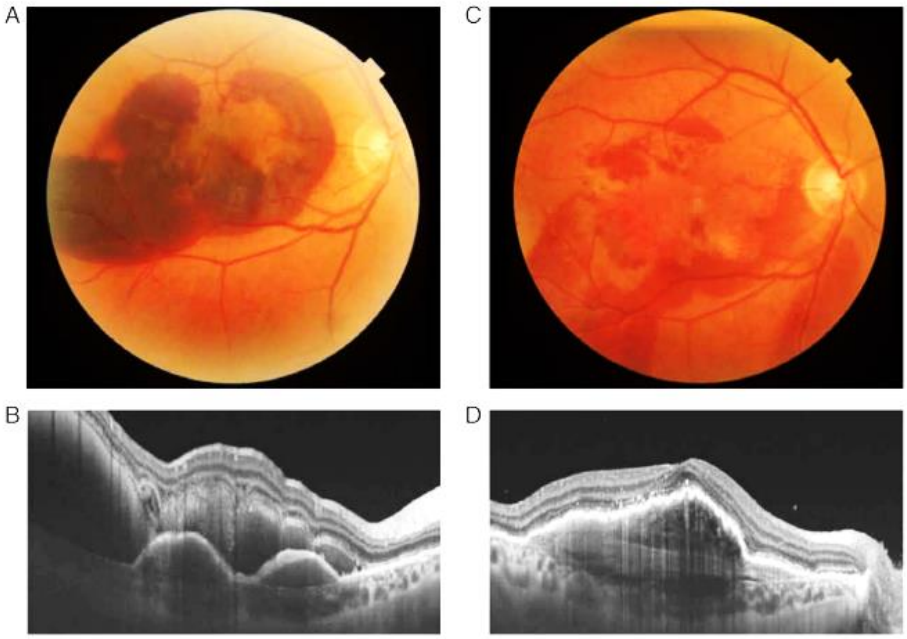


Figure 1.12 - Clinical and optical coherence tomography appearance of Case 1. (A and B) Fundus and optical coherence tomography (OCT) of the macula at the presentation, showed massive subretinal hemorrhage, significant retinal thickening, intraretinal fluid, moderate reflective material under neuroepithelium, and retinal pigment epithelial detachment (PED). (C) One month after surgery, almost complete resorption of the hemorrhage and the persistence of the air in the upper part. (D) OCT of the macula, one month after surgery, shows a significant decrease in retinal thickness, lack of subretinal blood, and the presence of a PED.

The second case is a 60-year-old male patient, without ophthalmological history, in treatment with oral anticoagulants for 3 years that was referred with acute vision loss for 2 days in the right eye. BCVA at presentation was hand motion. The funduscopic examination revealed an important SRH in the macular area, with a marked increase in retinal thickness on SD-OCT. PPV and subretinal rTPA injection were performed on the same day. Intraoperatively, a slight movement of the clot was already observed. Seven days after the surgery the BCVA was 0.05. The funduscopic aspect improved significantly, a few small areas of blood below the retina and under the RPE persisted. On SD-OCT the retinal thickness is close to normal, and foveolar depression is also observed. In one month no postoperative complications appeared, BCVA was 0.2, funduscopic and SD-OCT appearance were preserved or even small improvements were observed (Figure 1.13).

The third case is a 70-year-old woman with neovascular AMD, treated with anti-VEGF agents, and referred for a severe SRH. The last injection with bevacizumab was 10 months before this event. Visual acuity decreased to hand motion for about 4 days. The clinical aspect of the retina was similar to the previous cases, a massive submacular hemorrhage. On SD-OCT, subretinal and under RPE hemorrhages were observed. The same surgical steps were followed in this case. BCVA was counting fingers after the first week and 0.16 after one month, the patient was very happy that he returned to visual acuity as before the event. At the funduscopic examination, the remains of hemorrhage were visualized the first week postoperatively. The SRH disappeared almost completely over a month, and an area of RPE atrophy and pigmentary changes remained at the macula level. The SD-OCT showed an RPE detachment, with possible RPE rolls and a

significant distortion of the outer layers of the retina after 1 week. The aspect has improved significantly over a month (Figure 1.14). The patient followed regular check-ups, with intravitreal injections of bevacizumab or aflibercept as needed.

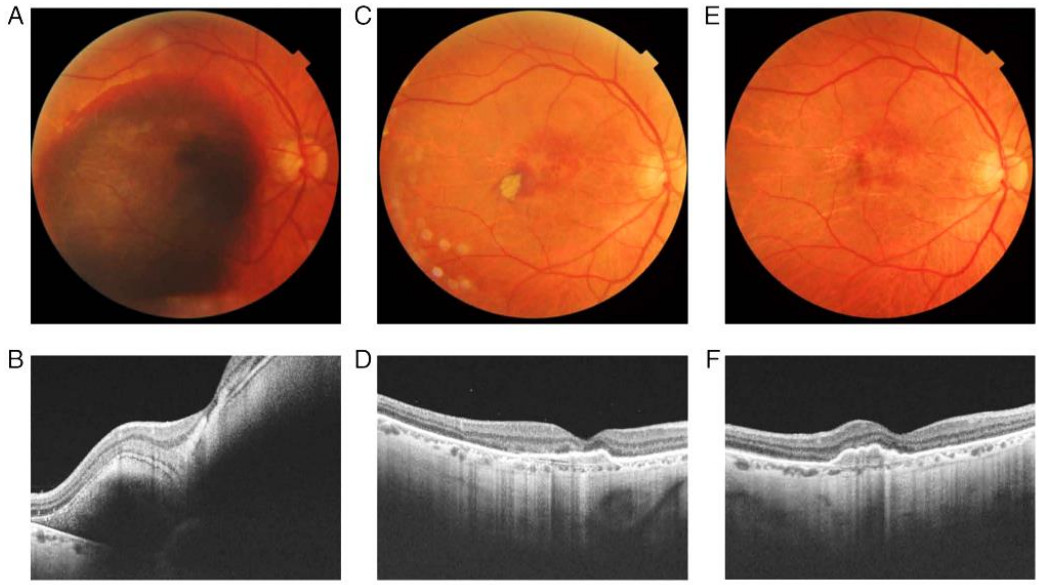


Figure 1.13 - Fundus and OCT at presentation, showing a massive subretinal hemorrhage, with a significant increase of the retinal thickness. (A and B) One week after surgery, the fundus shows significant improvement with the persistence of a small amount of dehemoglobinized blood, and significant reduction of retinal thickness on OCT. (C and D) One month after surgery, fine traces of subretinal hemorrhage, and persistence of RPE alterations on OCT (E and F)

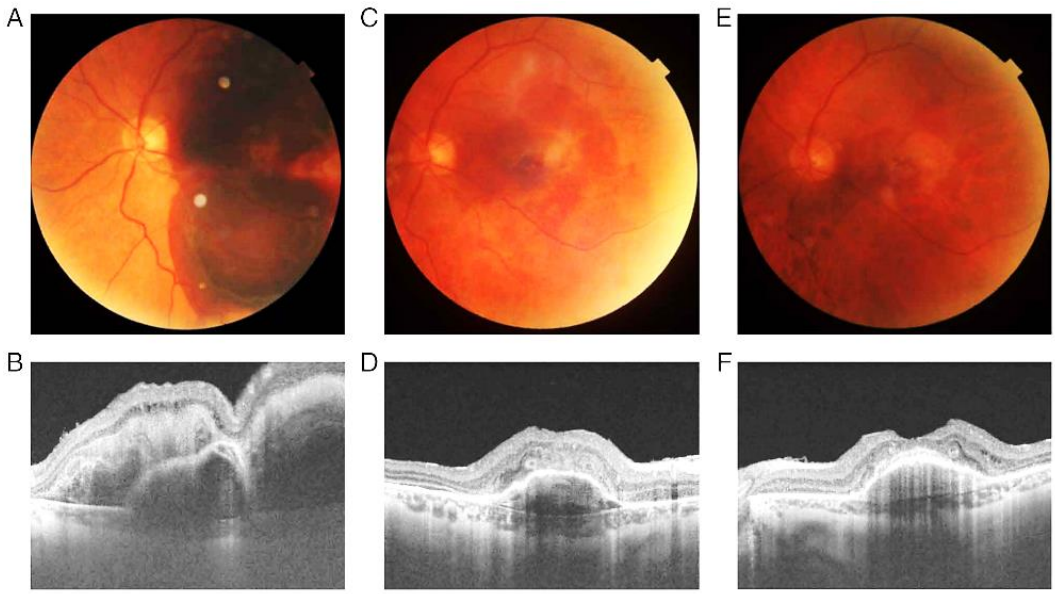


Fig 1.14 - Fundus and optical coherence tomography (OCT) at presentation, showed a massive subretinal hemorrhage, significant increase in the retinal thickness, subretinal hyperreflective tissue mass, and intraretinal cystic spaces. (A and B) One week after surgery, the persistence of subretinal blood in the macula but a significant decrease in retinal thickness, (C and D) One month after surgery, the hemorrhage disappeared almost completely, RPE detachment (E and F)

Regarding the intraocular pressure changes due to different endotamponade agents, raised IOP was reported soon after SIO started to be massively used in complex retinal detachments. Although the Silicone Study indicated an elevated IOP in only 8% of eyes with conventional SIO endotamponade after 36 months (Barr et al., 1993), literature data largely vary on this topic. Thus, in different reports, the percentage of elevated IOP or secondary glaucoma related to SIO endotamponade varies from 2.2 to 56.0% (Theelen et al., 2004, Honavar et al., 1999). Significant advances in vitrectomy technique and adjuncts purity explain, at least in part, the decreasing rate of secondary glaucoma in more recent reports.

Secondary glaucoma can develop in both the early and late postoperative stages of endotamponade (Jonas et al., 2001, Ichhpujani et al., 2009).

In the early postoperative period, the IOP elevation can be secondary to an overfill of silicone oil, a pupillary block, the migration of SIO into the anterior chamber, postoperative inflammation and/or steroid-induced ocular hypertension. The main risk factors for early secondary glaucoma are preexisting glaucoma, aphakia, iris neovascularization, and chronic uveitis.

In late postoperative stages, secondary glaucoma might occur due to a pupillary block, synechial angle closure, rubeosis iridis, and migration of non-emulsified SIO into the anterior chamber. Progressive SIO emulsification and migration of emulsified droplets into the anterior chamber are responsible for the chronic elevation of IOP.

The overfill of silicone oil is a frustrating situation responsible for an immediate increase of IOP and often requires partial removal of SIO as only a limited number of cases respond to lowering medication.

With an estimated incidence of around 1%, pupillary block glaucoma can develop in both the early and late postoperative period and is more frequent in the early stages in aphakic patients if a prophylactic inferior iridectomy has not been previously performed (25,26). The iridectomy must have between 150-200 microns, large enough to be efficient but not too large to allow forward migration of the oil. Late-onset pupillary glaucoma can occur anytime during endotamponade and is mainly related to the closure of existing peripheral iridectomies. As up to 35% of iridectomies progressively close in time, a strict follow-up is required and a YAG-Laser reopening or new iridectomy is necessary in these cases. If the laser treatment fails, a new surgery has to be performed.

In aphakic patients, but also in phakic and pseudophakic patients with disrupted lens zonule or capsular defects, an SIO bubble can migrate into the anterior chamber right at the end of surgery. This complication is easily prevented in aphakic and pseudophakic eyes by performing an inferior iridectomy during pars plana vitrectomy before SIO implantation. In phakic eyes, the migration of an SIO bubble into the anterior chamber at the end of surgery or later on is a challenging situation as SIO aspirated through paracentesis is quickly replaced from behind, sometimes resulting in more SIO in the anterior chamber.

Emulsification of the SIO is one of the most common causes of secondary glaucoma during endotamponade. The results of in vitro studies indicating that higher viscosity is associated with better long-term stability due to a lower rate of emulsification (Petersen et Ritzau-Tondrow, 1988) have been contested in real-life. The only randomized, double-blinded, controlled study to date, conducted on patients with complicated retinal detachments, has shown that low viscosity SIO has a non-significant higher rate of emulsification in short and medium-term (up to 6 months) but much higher emulsification rate in endotamponades over this time as compared with higher viscosity SIO (Ratanapakorn et al., 2020). Emulsification is not exclusively related to the physicochemical properties of SIO. As mentioned before, SIO contact with other

chemical compounds during surgery (such as PFCL) or with different biological emulsifiers inside the eye during endotamponade can precipitate emulsification. Also, eye movements induce a shear force on the SIO bubble that might enhance emulsification, especially in cases of SIO under-filling (Chan et al., 2014). The presence of an encircling band that provides indentation and reduces SIO velocity seems to provide a protective effect against emulsification (de Silva et al., 2005).

While emulsification time is believed to largely vary between 5 and 24 months postoperatively (Toklu et al., 2012), some reports emphasize that the first signs of SIO emulsification appear in the first 3 months postoperatively (Odrobina et Laudanska-Olszewska, 2014) and even earlier, in the first month postoperatively (Ratanapakorn et al., 2020). Since most of the cases have a certain degree of SIO emulsification within the first year postoperatively, there is a large consensus on the necessity to remove the SIO during this interval.

Once emulsification has started, small oil droplets migrate and induce complications in all ocular and extraocular structures. Secondary glaucoma, cataract, and keratopathy are the direct consequence of oil droplet interference with the metabolism of anterior segment structures. In the early stages, small droplets, like 'fish eggs' can be noticed in the anterior chamber or the angle. When emulsification is extensive, a typical image of 'inverse hypopyon' can be observed in the upper part of the anterior chamber. Despite this mechanical outflow obstruction, intraocular pressure remains normal in many cases (Nguyen et al., 1992). Further infiltration of micro-droplets into the trabecular meshwork is responsible for local inflammation (trabeculitis) and chronic IOP elevation (Riedel et al., 1990).

Secondary glaucoma during SIO endotamponade can be efficiently controlled in up to 80% of the cases with topical and systemic anti-glaucomatous medication. Topical aqueous suppressants are commonly recommended as first-line treatment as prostaglandin analogs might promote intraocular inflammation and cystoid macular edema (Alm et al., 2008). Topical cycloplegics and corticosteroids are recommended to decrease local inflammation in selected cases.

Removal of SIO with systematic irrigation of emulsified droplets from the anterior chamber offers a heterogeneous influence on intraocular pressure, largely ranging from normalization of IOP in >90% of cases (Jonas et al., 2001) to persistent IOP elevation in all cases (Flaxel et al., 2000). A more realistic outcome on PIO value is better to be expected later postoperatively, as small droplets and trabecular meshwork inflammation gradually disappear in time.

One complication related to SIO removal is the postoperative transient hypotony, with an incidence ranging between 5 and 40% of the cases (Song et al., 2010). An intraocular pressure less than 6 mmHg can induce various choroidal detachments that usually resolve spontaneously within 1 week with topical steroidal medication. While 23 G vitrectomy is associated with a lower incidence of postoperative hypotony, eyes with longer axial length are considered to be at risk (Kim et al., 2010).

Glaucoma surgery is required when significant trabecular meshwork damage has been produced. As conventional filtration surgery has a limited success rate in the management of secondary glaucoma, glaucoma drainage implants and cyclodestructive procedures have to be considered a better option (Al-Jazzaf et al., 2005, Bhoot et al., 2018). Mechanical angle closure due to iris tissue can be improved by surgical pupilloplasty with a single-pass four-throw technique (Narang et al., 2017).

In a retrospective evaluation of 98 consecutive cases with complex retinal detachments that underwent 23G vitrectomy and high viscosity SIO endotamponade (Oxane® 5700) we noted significant IOP changes during both SIO endotamponade and

after SIO removal (8). In 52 out of 98 cases (53.06%) with no preexisting glaucoma, IOP increased over 21 mmHg requiring topical antiglaucomatous medication during endotamponade. Most of the cases developed increased IOP during the first month (34 cases-65.38%). The main duration of SIO endotamponade was 5.46 months (3-16 months). An early postoperative hypotony was noted in 38 out of the 98 eyes (38.77%) after SIO removal, leading to transient choroidal detachments in 8 eyes (8.16%). IOP gradually decreased after SIO removal and at 12 months of follow-up, only 16 out of 98 eyes (16.32%) still required lowering medication. None of the cases required glaucoma surgery during or after SIO endotamponade.

In order to evaluate the **anatomical results and complications after silicone oil removal,** a total of consecutive 98 eyes from 98 patients were reviewed in a retrospective study. The mean age of patients was 53.7 years (28-72 years). The main indications for SIO endotamponade during ambulatory 23G vitrectomy were represented by proliferative vitreoretinopathy (PVR) (78 cases - 79.59%), proliferative diabetic retinopathy (17 cases - 17.34%), and giant retinal tears (3 cases - 3.06%). 29 eyes (29.59%) were pseudophakic at primary surgery. The main duration of oil endotamponade was 5.46 months (3–16 months). 51 out of 98 cases (52.04%) had the SIO removed within the first 6 months, 43 (43.87%) within the next 6 months and only 4 cases (4.08%) required maintaining the SIO endotamponade for more than 1 year up to removal moment. Signs of SIO emulsification were noted in 21 cases (21.42%), most of them (17 cases – 80.95%) after 6 months of endotamponade.

A significant number of eyes with no preexisting glaucoma developed increased intraocular pressure over 21 mmHg during SIO endotamponade (52 out of 98 cases - 53.06%) and required topical lowering medication. After SIO removal, the intraocular pressure decreased gradually and only 16 out of 98 eyes (16.32%) still required medication at 12 months of follow-up.

Anatomical success, defined as a stable attached retina at 12 months follow-up after SIO removal, was achieved in 94 out of 98 cases (95.91%) (Figures 1.15 and 1.16). Visual acuity increased with at least 2 lines in all cases.

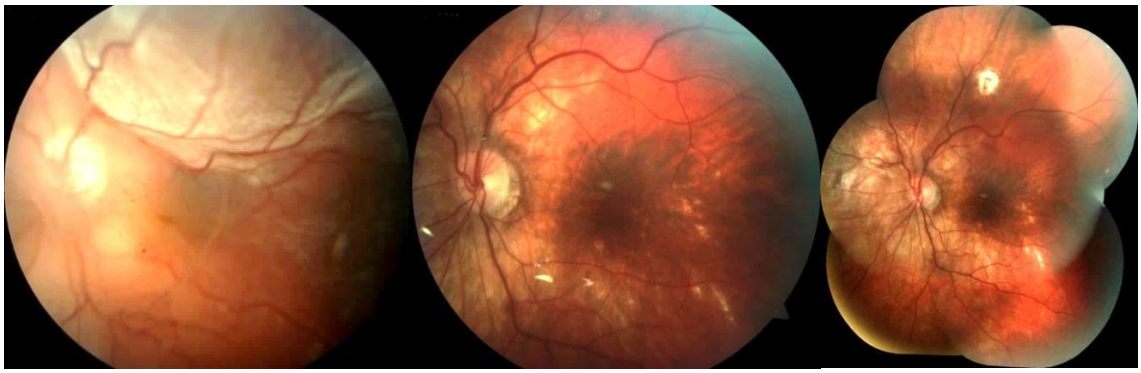


Figure 1.15 - Retinal detachment related to a large peripheral tear.
Left image: before surgery; Middle image: during SIO endotamponade;
Right image: 12 months after SIO removal.

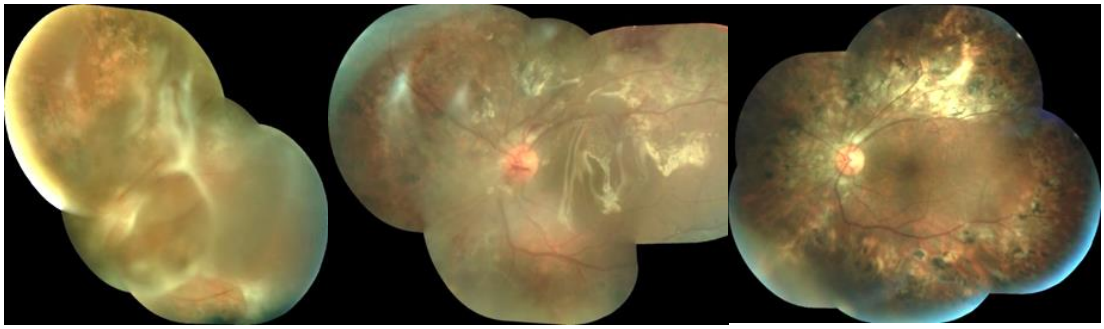


Figure 1.16 - Mixed retinal detachment in proliferative diabetic retinopathy.
Left image: before surgery; Middle image: during SIO endotamponade;
Right image: 12 months after SIO removal.

Retinal detachment recurrence occurred in 4 cases, one in the first month postoperatively and the other 3 cases between the 3rd and 4th months. One of the patients had diabetic retinopathy, and the other 3 were operated on for proliferative vitreoretinopathy in the presence of high myopia.

No intraoperative complications were encountered at the time of SIO removal.

Transient hypotony affected 38 out of the 98 eyes (38.77%) the next day after SIO removal and 8 eyes (8.16%) developed various choroidal detachments resolving spontaneously within the first week postoperatively (Figure 1.17).

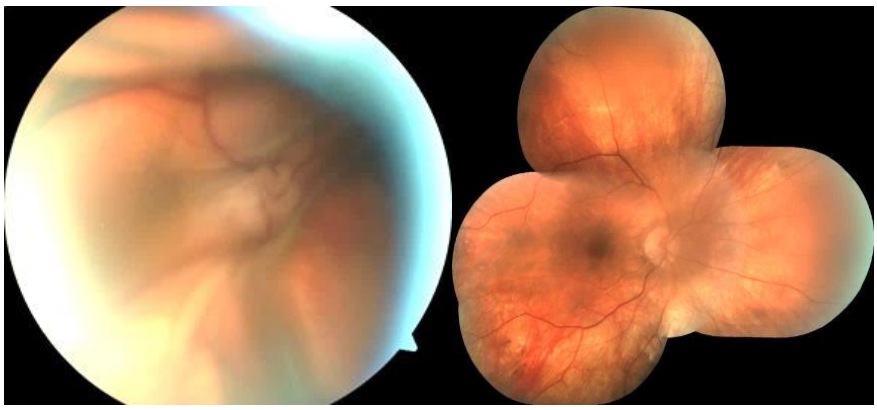


Figure 1.17 - Massive choroidal detachment due to postoperative hypotony.
Left image: at 24 hours after SIO removal;
Right image: detachment completely resolved within 7 days postoperatively;

Most phakic eyes showed cataract development and progression during both SIO endotamponade and after SIO removal. At 12 months follow-up, 47 out of 69 phakic patients (68.11%) required cataract surgery. At the last follow-up, 4 cases (4.08%) presented small residual asymptomatic silicone bubbles in the vitreous cavity.

The study on **vitrectomy surgery of diabetic retinopathy complications** involved 1267 eyes of 1129 patients who were operated on for different complications of diabetic retinopathy during 15 years' experience. According to the authors' knowledge, this was the largest series described in Romanian literature at that time. Among these patients, 540 were men and 589 were women, the ratio between the two being statistically insignificant (0.916).

The mean age of patients was 57.49 years ± 14.17 years (with limits between 16 and 78 years old). The majority of patients, 864 (76.52%), had type 2 diabetes. Of 1129 patients included in this study, 832 (73.69%) had one or more associated systemic conditions as detailed in Figure 1.18. The most frequent associated conditions were arterial hypertension (49.95%), cardiac failure (15.85%), and diabetic nephropathy (15.94%).

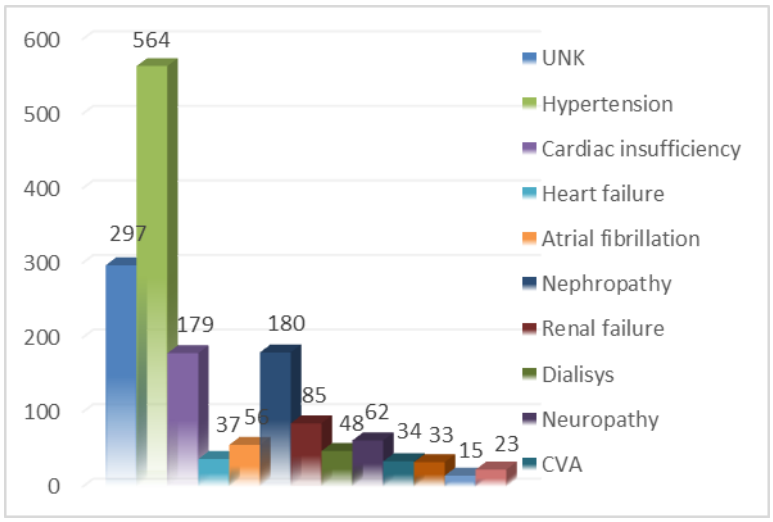


Figure 1.18 - Associated systemic conditions.

The main indications for surgery were vitreous opacities (609 cases – 48.06%), vitreoretinal tractions, and retinal detachments (583 cases – 46.01%) (Figure 1.19). The other indications included: persistent retinal neovascularization with rubeosis iridis (37 cases – 2.93%), and persistent macular edema (38 cases – 3%).

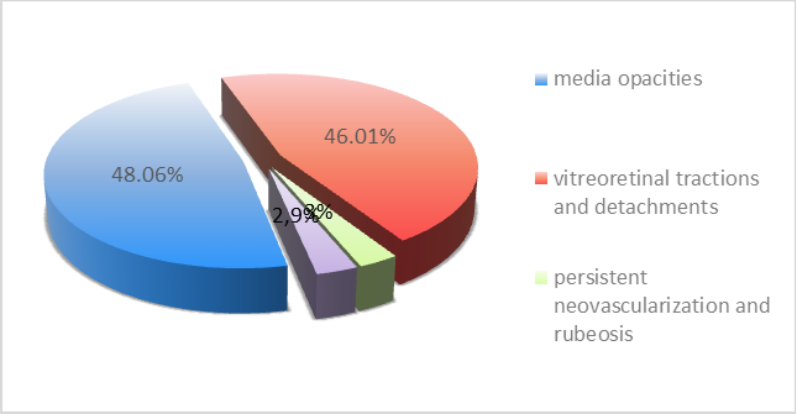


Figure 1.19 - Distribution of cases according to surgical indication.

The standard 20G vitrectomy was performed in 689 cases (54.38%), and transconjunctival 23G was performed in 578 cases (45.61%).

Most cases (1124 – 88.71%) had a stable anatomical result after the initial surgery (Figures 1.20, 1.21 and 1.22). With repeated surgical interventions, a final anatomical success was recorded in 1174 cases (92.65%). 93 eyes (7.34%) were finally lost due to extensive complications. Preoperative BCVA was less than counting fingers (0.002) in 936 cases (73.87%). Postoperatively, the BCVA improved in 923 cases (72.84%),

stabilized in 201 cases (15.86%), and decreased in 143 cases (11.28%). At the last follow-up, 932 eyes (73.55%) had a BCVA of ≥ 0.1 and a mean of 0.21 ± 0.16. Cases that underwent a 23G surgery and the cases that were operated on for vitreous opacities or tractions not involving the macula had a better anatomical and functional prognosis.

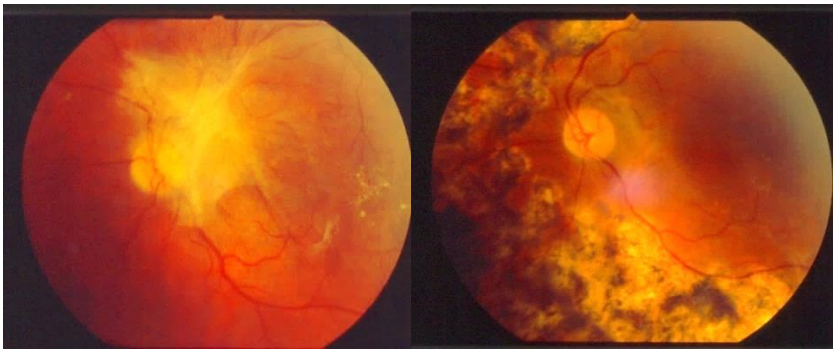


Figure 1.20 - Fibrovascular membrane with macular involvement. Pre and 12 months postoperative 20G vitrectomy (2003); BCVA improved from 0.1 to 0.5.

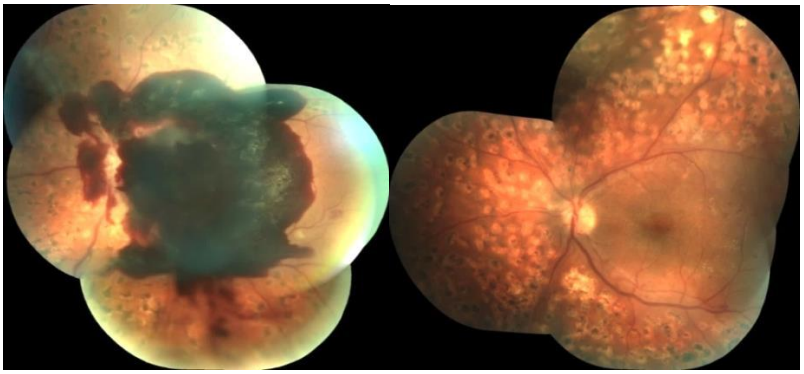


Figure 1.21 - Massive preretinal hemorrhage: Pre and next day postoperative 23G vitrectomy (2013) BCVA improved from 01. to 0.5.

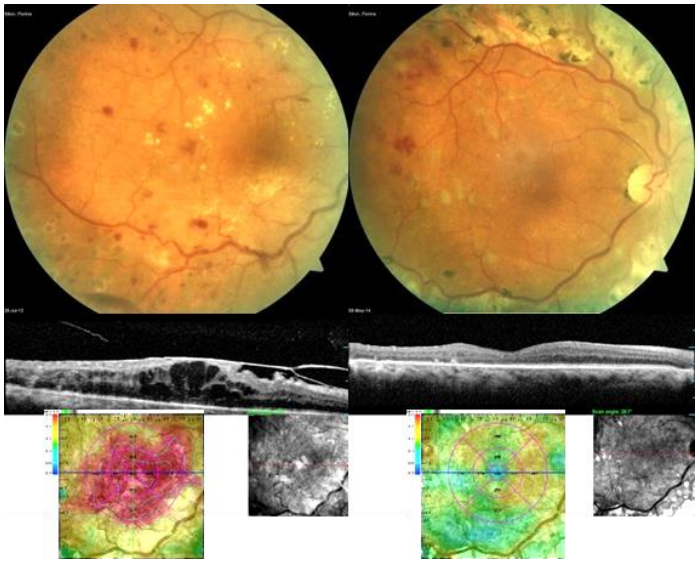


Figure 1.22 - Tractional macular edema. Pre and 6 months postoperative 23G vitrectomy (2014); BCVA improved from 0.1 to 0.7.

The main intra and postoperative complications encountered were iatrogenic breaks, cataract, recurrent hemorrhage, recurrent retinal detachment due to proliferation, and neovascular glaucoma. With the conversion to the 23G technique, the number of iatrogenic breaks and recurrent retinal detachments significantly decreased. Also, the intraoperative use of anti-VEGF in selected cases significantly decreased the risks of progression to neovascular glaucoma and bleeding. Systemic complications were noticed intraoperatively in only two cases: one case of hemorrhagic stroke and one case of ischemic stroke. Only the latest, fully recovered.

1.5.5. Discussions

Regarding **cataract surgery** during but especially after PPV, it may represent a challenge even for the most experienced surgeons. A vitrectomized eye presents with capsular damage and zonular weakness, a harder nucleus, and unstable dynamics of the anterior chamber rendering the intervention more difficult than in routine cases. The advantage of a combined procedure, besides the economic factors involved, is the clear eye media that allows a safer surgery, provides the ability to shave the vitreous base, especially during retinal detachment surgery, to recognize and treat an iatrogenic break and avoid the risk of touching the lens with the vitreoretinal instruments. Cataract surgery cannot be avoided in most PPV patients, as Braunstein and Airiani reported that 80% of these patients develop lens opacification during the first 2 years. Also, the combined procedure allows a quicker rehabilitation and socio-economical reintegration of the patient.

However, there are some shortcomings related to performing simultaneous surgeries. Besides a prolonged procedure, it is expected to have a higher ratio of anterior segment inflammation and synechia formation postoperative, by comparison to sequential surgery. There is a higher rate of capsular rupture and difficulty in accomplishing correctly the surgical steps due to depth variations of the anterior chamber, absence of red reflex, or posterior displacement of the irido-lenticular diaphragm.

Analyzing the data obtained in this study, we observed that there are no major differences between the posterior capsular rupture rates between the two groups, nor between the intraoperative miosis rates. Both are higher in group 2, which benefited from sequential surgery, but there were more patients in this group. Miosis may be related to a variety of causes including prolonged surgery, surgical trauma, but also previous medication administered to the patient (especially α-blockers), pseudoexfoliation syndrome and in all cases responded well to intracameral epinephrine 0.001%. There were more eyes with intraoperative posterior capsular rupture in group 2, 15.21% as compared with 7.31% in group 1, most of them related to anterior chamber imbalance in silicone oil-filled eyes, or a weaker zonule in the vitrectomized eyes. Although the percentage of intraoperative posterior capsular rupture in the sequential surgery group is higher, the number of eyes affected by this incident was low, 3 in group 1 vs. 7 in group 2.

Another concern while performing the combined procedure is the maintenance of the anterior chamber depth, which requires the use of a higher quantity of viscoelastic and in some cases the incomplete removal of the OVD at the end of the surgery. Also, IOL decentration, transfer of silicone oil or air/gas from the vitreous cavity to the anterior chamber, and visualization issues during vitrectomy due to endothelial stria or the IOL margins may render the combined procedure more difficult.

Regarding the best time for IOL implantation in the combined procedure, there are some controversies. Some authors consider that it is preferable to inject the IOL at the end

of the surgery, when both cataract and vitrectomy were resolved, thus avoiding the prismatic effect of the IOL edge that hampers the vitreous base vitrectomy (Hurley et al., 1996). Other studies demonstrate that there is a lower risk of damaging the posterior capsule if the IOL is injected at the end of the cataract surgery and before PPV, the approach that was preferred in all cases included in group 1 in our study (Jain et al.,2007).

Simultaneous surgery is considered to have a higher risk of postoperative inflammation (Aptel et al., 2017). Recent literature data reported rates of inflammatory reaction and fibrin formation in the anterior chamber after combined surgery comprised between 3.5-20% (Wensheng et al., 2009, Tosi et al., 2017, Tayyab et al., 2017). The progress in instrumentation recorded during the last decade seems to have an important role in decreasing these rates. Earlier studies considered that the intact lens may act as a barrier, preventing the transfer of inflammatory factors between the anterior chamber and the vitreous cavity. In recent years, the levels of cytokines and growth factors were measured and reported at high values in the anterior chamber after cataract surgery and in the vitreous cavity after PPV (Jakobsson et al., 2015, Iyengar et al., 2009). Iris manipulation during anterior segment procedures presents an additional risk of inflammation with high levels of IL-1, IL-8, IL-0, IL-6, and TNF-α, cytokines that are proven to be involved in the development of proliferative vitreoretinopathy (Moysidis et al., 2012). Also, in vitrectomized eyes, the inflammatory cytokines are present many months after PPV (Gu et al., 2016). These factors are known to promote fibrosis development in the form of proliferative vitreoretinopathy and posterior capsule opacification, which explains why there is a higher inflammatory risk when the procedures are accomplished simultaneously. In our study, the reoperation rate was higher in group 1, due to the occurrence of retinal redetachment in more eyes as compared with group 2, which can be explained by the breakdown of both the blood-aqueous barrier and the blood-retina barrier at the same time. The redetachment of the retina is also a factor that contributes additionally to the inflammatory cascade by releasing supplementary cytokines and pigment epithelium cells. When considering the percentage of reoperated patients due to redetachment, the values obtained in this retrospective study are comparable to literature data (Loukovaara et Haukka, 2018, Park et al., 2009). However, some reinterventions were effectuated for reasons not related to the inflammatory process, such as IOL displacement or vitreous hemorrhage. One limitation of this study is the fact that we did not take into account the number of patients that needed reintervention due to secondary epiretinal membrane occurrence. In all cases, the anterior segment fibrinous reaction responded well to intensive local anti-inflammatory therapy, so this episode did not influence the final visual outcome.

The posterior capsule opacification rate was higher in group 1 (17.07%) in comparison to group 2 (10.87%). It is postulated that long-term endotamponade and inflammation contribute to a more frequent occurrence of PCO in these eyes (Jalil et al., 2014). However, there is not a significant difference between the number of PCO cases in the two groups. Some eyes from group 2 suffered from an extended period of inflammation due to redetachment of the retina, hemorrhage, or were operated for cataract while the endotamponade was in place.

The eyes included in group 1 were more prone to develop postoperative corneal edema, due to a prolonged and more complex surgical intervention. In both groups, the edema resolved during the first postoperative week with topical treatment. Nor the PCO rate, nor the corneal edema influenced the final BCVA, as in both groups it was comprised between 0.3 and 0.5 in the majority of the cases. The mean IOP values were higher in group 1 during the immediate postoperative period, but at the end of the follow-up time, the mean values were similar between the two groups. Postoperative

inflammation and some difficulty in removing the viscoelastic from the eye during the combined procedure may have influenced this number. Regarding the effect of the silicone oil tamponade on IOP values, there was not a significant difference in the number of silicone oil-filled eyes in the two groups (16 from group 1 and 25 from group 2), nor in the period of endotamponade maintenance, which varied between 3 and 6 months.

AMD is one of the most common causes of SRH in adults. Therefore the definitive visual acuity of each patient, regardless of the therapeutic solution chosen, also depends on the degree of macular damage before bleeding.

The prognostic factors of postoperative visual acuity were observed to be visual acuity before hemorrhage occurred and the time elapsed since the onset of the SRH. The most frequent contraindication for rTPA injection was a late presentation. Late presentation means poor visual potential due to neurosensory retinal distress. All cases in which a visual acuity greater than counting fingers was obtained, were presented in the first 5 days of onset. The maximum duration of the SRH for which we used this technique was 3 weeks, and postoperative visual acuity was counting fingers at 1 m. We did not have any intraoperative complications due to alteplase or other factors. Regarding postoperative complications only one case of rhegmatogenous retinal detachment, caused by a peripheral retinal break, was observed.

Regarding the surgical technique used, we considered that it is one of the most optimal in terms of the applications during the surgery and after it. Probably one of the most widely used methods is for the surgeon to hold the syringe and an assistant to push the plunger. In other words, this technique requires a good collaboration between team members and good training of the entire team of surgeons and assistants. Another method to inject the alteplase is using the viscous fluid control unit. This is a semiautomated technique that requires an insulin syringe of 1-ml adapted to the viscous fluid control unit and a 41-gauge cannula. This technique probably offers more control and stability than the assisted one. In our opinion what makes our technique more practical and easy to use is that all operative steps can be performed by a single surgeon (Haupert et al., 2001). No additional time is needed for clot lysis or to prepare special injection devices (Novelli et al., 2017). There are no studies that compare the functional results of these surgical techniques, so at the moment the choice is strictly related to the comfort and possibilities of the surgeon. Based on our experience the fluid-air exchange is sufficient for the mobilization of the clot, the injection of an expandable gas is not necessary. The decision to inject an anti-VEGF drug at the end of the operation seems to be quite important, considering the pathophysiological mechanism of the SRH.

In massive hemorrhages, a larger amount of alteplase was needed for clot dislocation and dissolution. However, even in these patients, no dose-dependent toxic effect was observed. Also, no special attitude was taken towards patients treated with anticoagulants or anti-aggregating agents. All the patients followed the treatment recommended by the cardiologist. Moreover, the injection with an anti-VEGF agent at the end of the surgery had exactly this purpose, to prevent subsequent bleeding, which did not occur in any of the cases. Studying the maximum duration of the SRH for which surgery is worthwhile is one of our future goals. Another purpose is a long-term follow-up of patients in whom the subretinal alteplase was injected and their comparison with those in whom a less invasive therapeutic solution was decided.

Literature data does not provide precise timing for **SIO removal**. While early removal might impact the retinal stability, a late removal might be difficult because of associated SIO complications. A median 30 months endotamponade is responsible for

significant ocular complications, such as optic atrophy (28%), corneal decompensation (12%), band keratopathy (8%), and rubeosis iridis (14%) (Morphis, 2012, Miller et al., 2014). One of the most fearful postoperative complication after SIO removal is the recurrence of retinal detachment. According to literature, statistically significant risk factors for redetachment are the quality of primary surgery (incomplete removal of the vitreous base, previous number of retinal surgeries, surgeon's experience), the severity of initial PVR, and vitreous hemorrhage appearance in the first 3 days after SIO removal. PVR is associated with the highest risk of retinal redetachment, whereas diabetic retinopathy, high myopia, and giant retinal tears with a lower risk of redetachment (Jonas et al., 2001). Surprisingly, the technique of SIO removal and the duration of the SIO endotamponade were found of no prognostic significance concerning retinal redetachment by certain authors (Tan et al., 2012).

The retinal detachment recurrence rate of 4.08% achieved in our series is comparable to the most optimistic data of 3.46%. In order to avoid retinal redetachment, an accurate primary surgery is mandatory.

A common complication encountered during SIO removal is early hypotony. In our series, a transient hypotony was noted in 38 out of the 98 eyes (38.77%) the next day after SIO removal. All 8 eyes (8.16%) that additionally developed choroidal detachment evolved favorably with spontaneous recovery within the first week postoperatively. Literature data points out that the incidence of postoperative hypotony after SIO removal varies between 25% and 40% (Song et al., 201). Risk factors for early hypotony are a longer axial length (Kim et al., 2010), infusion cannula retraction (Tarantola et al., 2011), and incorrect trocar insertion. Apparently, with a 23 G system, there is a lower risk of hypotony as compared to other small G sutureless vitrectomies. Chen et al., pointed out that gaping, misalignment, and important variation in incision angle might have a negative impact on sutureless sclerotomy (Chen et al., 2010).

When removing SIO, it is recommended to be removed as completely as possible. Small silicone droplets can remain somehow attached to the trocar or on the inner surface of the retina and move freely within the vitreous cavity after that. Modern equipment can facilitate total removal and provide efficient active aspiration of high-density SIO even through small trocars.

The key to a lower rate of retinal detachment recurrence after SIO removal relies on both initial pathology severity and also on primary surgery quality. Careful removal of vitreous base and peeling of epiretinal membranes, sufficient laser retinopexy, use of relaxing retinotomies in advanced PVR, and complete SIO filling are correlated with a lower incidence of retinal redetachment.

Strict glycemic control and correction of associated conditions are mandatory to reduce the incidence of **surgery in diabetic retinopathy**, and also to provide better anatomical and functional results postoperatively. The standard follow-up protocol of diabetic patients has an important role in the early diagnosis and prevention of ocular complications. Prompt pan-retinal photocoagulation should be immediately performed in proliferative or severe non-proliferative diabetic retinopathy (Liew et al., 2009).

Pars plana vitrectomy has proved a standard of care for complications due to diabetic retinopathy cases that have been registered in the last decades. During the surgical intervention, the laser photocoagulation on the retina is completed, and, in selected cases, the intravitreal injection of anti-VEGF drugs or steroids helps reduce the angiogenesis and macular edema before, intra, or postoperatively.

The development of minimally invasive vitrectomy and the integration of 23G, 25G, and 27G systems into current clinical practice has led to a much more efficient,

quicker, and safer procedure. The transconjunctival sutureless approach has spectacularly improved the patient's comfort and recovery. Smaller instruments and high cutting probes make small G vitrectomy highly efficient even in the most complicated cases. Unlike the 20G vitrectomy probe, in the small G technique, the vitrector can be used as a multifunctioning tool for cutting, segmenting, dissecting, and removing the fibrovascular membrane, as well as for aspirating blood or subretinal fluid. Numerous studies confirmed that small G vitrectomy provides better anatomical and functional results also due to reduced postoperative inflammation (Gupta et al., 2012, Goldenberg et al., 2009).

The integration of SD-OCT in our current clinical practice and the use of intravitreal anti-VEGF drugs since 2007 have also changed the approach to diabetic retinopathy complications. SD-OCT offers high-resolution, cross-section images of the macula in a quick, non-invasive way. Thus, the macular thickness, the morphological structure of all layers, and the vitreoretinal interface can be evaluated. We are now able to make a clear distinction between different types of macular edema and immediately refer to surgery in those cases with obvious macular traction (Figure 22).

Most studies proved that the intravitreal administrations of anti-VEGF agents in the cases of proliferative diabetic retinopathy, persistent neovascularization and rubeosis iridis, significantly decrease neovascularization and improve macular edema (Harrison et al., 2013). Still, the intravitreal administration of VEGF inhibitors did not become a standard of care in proliferative diabetic retinopathy and its complications because requires frequent administration and monitoring. Anti-VEGF intravitreal administration is also associated with systemic risks. The reoccurrence of proliferation, membrane contraction, and worsening of the retinal traction are some of the reported ocular side effects.

The indications for vitrectomy in macular edema have changed in the last decade due to the use of anti-VEGF agents and SD-OCT. Still, the anti-VEGF brings no benefit in cases of tractional macular edema, in which surgery may be mandatory. Also, surgery is indicated in macular edema refractory to multiple intravitreal anti-VEGF or steroids administrations because it improves oxygen diffusion from the vitreous to the retina and decreases the quantity of intraocular VEGF.

Despite the major innovations in diabetic retinopathy treatment, most ocular complications are still resolved by vitrectomy: non-clearing vitreous hemorrhage, tractional or combined retinal detachment, severe fibrovascular proliferation, macular heterotopia, and tractional diabetic macular edema.

The DRVS study confirmed the benefits of early vitrectomy, significantly more patients who underwent an early surgery had a better final BCVA and stable results after 4 years. Nowadays, the proper time of surgery is individually established according to the status of the fellow eye, the degree of visual impairment, the presence of associated ocular findings, and the lifestyle of the patient.

The most frequent intraoperative complications associated with diabetic vitrectomy are iatrogenic retinal breaks and hemorrhages. The iatrogenic breaks mostly occur in the thin or atrophic retina during membrane peeling, close to the tractions, and have to be properly lasered all around. Hemorrhages are rare due to direct vascular injury, but more often because of neovascular peg segmentation, and are easily controlled by diathermy. The intravitreal anti-VEGF administration a few days before surgery is a useful manner to reduce the intraoperative bleeding in the eyes with extensive neovascularization.

The most frequent postoperative complications are cataract, recurrent hemorrhage (17-26%, with a higher frequency in younger patients), rubeosis iridis, and neovascular glaucoma (Oshima et al., 2009). The intraoperative administration of anti-VEGF drugs at

the end of surgery is an easy gesture to prevent uncontrolled postoperative angiogenesis and severe complications.

The results obtained in our study confirmed the reported literature data on a significant number of cases. A careful vitrectomy with a complete membrane removal and intraoperative photocoagulation leads to a good anatomical and visual result in most cases. The vast majorities of cases remain stable and do not require additional surgery.

Although performed on a smaller number of patients (because of a later integration in clinical practice), the minimally invasive small G vitrectomy proved to be an excellent tool for ambulatory surgery due to its higher facility, excellent efficacy and safety, and faster recovery of the patient.

1.5.6. Conclusions

Combined pars plana vitrectomy and cataract surgery is safe and effective in obtaining good morphological and functional results in this category of patients. Although the difficulty of the surgical procedure and the number of adverse reactions were higher in the group that underwent the simultaneous surgery, the rehabilitation was quicker for these patients and the final functional results were comparable between the two groups. The analysis of the risks and benefits of each procedure should be taken into account and the cases selected individually for either simultaneous or sequential surgery in order to obtain the best outcomes.

SRH is a common complication of AMD which can lead to irreversible loss of visual acuity. Due to intravitreal injections with anti-VEGF agents, AMD in most patients can be kept under control. Thus, surgery has become an indication only for complicated cases of SRH. **Subretinal injection with rTPA** appears to be a viable solution in patients with massive SRH who are addressed on time and should be considered as the first therapeutic approach.

Silicone oil endotamponade is an important risk factor for IOP changes after pars plana vitrectomy in a percentage that largely varies. Among all mechanisms of secondary glaucoma development, pupillary block and anterior migration of emulsified or non-emulsified SIO are the most frequent. A careful follow-up is mandatory during endotamponade as a rise in IOP can occur at any time. Prompt SIO removal at first signs of emulsification does not guarantee IOP restoration to normal values in all cases. Also, SIO removal can be followed by significant hypotony, usually transient but responsible for choroidal detachments. Chronic elevation of IOP in advanced stages, regardless of the medical treatment, requires complex glaucoma surgery.

The retrospective evaluation of retinal detachment recurrence rate after **SIO removal** in a personal series was 4.08%. This promising anatomical result confirms the need for an accurate primary surgery and also for choosing a safe moment for SIO removal according to the severity of primary pathology.

In my 15 years of experience, **vitreoretinal surgery proved to be efficient and safe in improving most complications due to diabetic retinopathy.** The new 23G transconjunctival vitrectomy has enhanced feasibility as ambulatory surgery, offers a higher efficacy and comfort, and allows a faster patient recovery. Many complications of diabetic retinopathy are now medically treated, but the most severe ones still require the surgeon’s skills and state-of-the-art equipment.

Chapter2. Anti-VEGF agents: the revolutionary treatment paradigm shift in neovascular retinal diseases

2.1. State of the art and scientific context

There is no other therapy in the last few decades to have such a significant impact on the world of retinology as the introduction of anti-VEGF agents.

It all started back in 1948 when scientists supposed that a mysterious "Factor X", as termed by George Wise, is produced by the retina in multiple ischemic retinopathies, and is responsible for the growth of new blood vessels. The answer came 50 years later when the vascular endothelial growth factor (VEGF) was discovered by Napoleone Ferrara and thus, his major role in neovascular retinal pathology and macular edema was revealed. Napoleone Ferrara is also the father of the first approved anti-VEGF agent, bevacizumab (Avastin®), in 2004, a humanized anti-VEGF antibody designed to block all VEGF isoforms and indicated in colon cancer. At that time, the first reports about the concomitant improvement of the neovascular AMD status in patients with colon cancer systemically treated with Avastin® were published. Moreover, an increasing number of cases with significant neovascular AMD improvement after intravitreal injection of a small amount of Avastin® were reported worldwide.

I have witnessed, at different international congresses, these first promising reports from 2005 and 2006 on the bevacizumab "off-label" usage in intravitreal administration in neovascular age-related macular degeneration. Upon my return, I have personally performed, in the early 2007 first treatments with intravitreal bevacizumab in Iasi, on the same "off-label" basis, thus succeeding in implementing this revolutionary technique in the current clinical practice and saving vision in many hundreds of patients.

During the following years, the anti-VEGF development process quickly evolved with new fascinating innovations in the pipeline for neovascular AMD. Although pegaptanib (Macugen®), was the first approved anti-VEGF agent with this indication, the modest results in the current practice discontinued its usage. Ranibizumab (Lucentis®), a small fragment from bevacizumab, was the first anti-VEGF agent able to neutralize all VEGF-A isoforms and restore significant vision in many patients, thus dramatically reducing the risk of progression to blindness. Later on, Ranibizumab became the first anti-VEGF agent approved for the treatment of retinal venous occlusions, diabetic macular edema, and then diabetic retinopathy itself. Afliberecept (Eylea®), succeeded ranibizumab with these indications and because inhibits both VEGF-A and the placental growth factor is recommended once every 2 months after the loading phase. The recent approval of Brolucizumab (Beovu®), and a port delivery system (PDS) with ranibizumab (Susvimo®) are meant not to only diversify the market but to allow a significant reduction of the treatment burden as all of these newer agents are needed less frequent to be administered.

Still, numerous trials have suggested that the anti-VEGF therapy could have already reached an efficacy ceiling as increased potency and/or higher doses do not further improve final vision. Thus, targeting a secondary mechanism together with the VEGF might improve both the efficacy and also persistence of the result. The recent approval of Faricimab (Vabysmo®), the first bispecific antibody, neutralizing both angiopoietin-2 (Ang-2) and vascular endothelial growth factor-A (VEGF-A) has demonstrated complementary benefits in reducing leakage and also inflammation.

Although injected in a small amount into the vitreous cavity, the introduction of additional fluid into the vitreous cavity can cause an immediate rise in intraocular pressure (IOP). Also, a systemic passage of different anti-VEGF agents has been already

demonstrated as well as their influence on the fellow eye. To date, the literature data on these topics is controversial. The repeated intravitreal injection increases the risks of specific complications related to both the substance and also to the procedure. Among them, an increase in intraocular pressure has recently been noticed.

Iasi is one of the first medical centers in Romania where intravitreal anti-VEGF therapy has been implemented. For more than a decade, with very small exceptions, Avastin® was the only drug available and largely used on an "off-label" basis. With no exception, all of the international comparative studies performed during the years proved no major differences in efficacy and safety between bevacizumab, ranibizumab, and aflibercept. Bevacizumab is significantly cheaper than the other agents but despite its comparable properties it continues to be used on an "off-label" basis, especially in developing economies. Fortunately, for a couple of years, aflibercept became largely available and fully reimbursed in Romania. This year, brolucizumab will also be available.

In these conditions, it is easily understandable why most of the Romanian literature on the topic is related to the usage of Avastin®.

This direction of research is reflected in the following published articles:

Branisteanu DC, Branisteanu DE, Feraru CI, Branisteanu CI, Moraru A, Zemba M, Balta F. Influence of unilateral intravitreal bevacizumab injection on the incidence of symptomatic choroidal neovascularization in the fellow eye in patients with neovascular age-related macular degeneration (Review), Experimental and Therapeutic Medicine, 2020, 20(6): art. no 182. **IF=1.785**

https://doi.org/10.3892/etm.2020.9312

Branisteanu DC, Munteanu M, Branisteanu DE, Stanca HT, Moraru A, Balta F. «Off-label» Drug use - Ethical challenges – Case Study - AVASTIN, Revista Romana de Bioetica, 2015, 13(3): 1-6 (Scopus)

Moraru A, Pinzaru G, Motoc A, Costin D, **Branisteanu D.** Incidence of ocular hypertension after intravitreal injection of anti-VEGF agents in the treatment of neovascular AMD, Romanian Journal of Ophthalmology, 2017, 61(3): 207-211 (Pubmed)

Branisteanu DC, Bilha A, Moraru A. Aflibercept efficacy in refractory choroidal neovascularization, Romanian Journal of Ophthalmology, 2016, 60(2): 96-102 (Pubmed)

Branisteanu D, Moraru A. The results of intravitreal bevacizumab in subretinal neovascularisation in angioid streaks, Oftalmologia, 2014, 58(1): 48-55 (Pubmed)

Branisteanu D, Moraru A. The results of intravitreal bevacizumab in high myopic subretinal neovascularisation, Oftalmologia, 2013, 57(3): 58-65 (Pubmed)

2.2.2. Aim

Neovascular age-related macular degeneration (neovascular AMD) represents only 10% of AMD cases but is responsible, if untreated, for quick and severe central vision loss due to major macular changes. The presence of choroidal neovascularization (CNV) in one eye is associated with an approximately 10% risk of CNV development in the fellow eye each year. Intravitreal anti-VEGF therapy has quickly evolved as the standard treatment in neovascular AMD in the last decade due to significant anatomical and functional improvements, especially in the early stages. In many reports, an improvement in the untreated fellow eye was mentioned and systemic exposure was soon confirmed for all anti-VEGF agents after unilateral intravitreal injection. In particular, bevacizumab intravitreal injection is followed by a consistent reduction of serum VEGF levels and the drug was shown to have the longest serum half-life raising important debates about its safety. Once bevacizumab was detected in the fellow eye of an animal model after unilateral injection, the possible influence on fellow eye conversion rate into neovascular AMD was questioned. The review aims to summarize the most recent data concerning the incidence of symptomatic CNV in the fellow eye during treatment. Also, to evaluate, on a retrospective 36-month evaluation, the personal experience on the topic.

Due to their remarkable ability to quickly improve symptoms and also to provide consistent visual recovery, intravitreal administration of different anti-VEGF agents is nowadays the standard treatment for many retinal diseases including neovascular AMD, diabetic retinopathy, diabetic macular edema, venous occlusions, and other retinal disorders (Villegas et al., 2017). Ranibizumab (Lucentis®) and Aflibercept (Eylea®) are FDA and EMA approved for intravitreal use in neovascular AMD and recently Brolucizumab (Beovu®) received both approvals for this indication. Although multiple studies have confirmed the comparable efficacy and safety of intravitreal bevacizumab (Avastin®) administration to registered anti-VEGF drugs (Chakravarthy et al., 2013, Martin et al., 2012, Schawvlieghe et al., 2016, Krebs et al., 2013, Kodjikian et al., 2013, Berg et al., 2015), bevacizumab continues to have an off-label status. A recent study evaluating bevacizumab use in 20 European countries showed that a consensus on the ophthalmic off-label use of bevacizumab in Europe has not yet been reached and member states have different approaches (Bro et al., 2020). Bevacizumab intravitreal injections quota significantly varies in different European countries and even developed economies use bevacizumab after informed consent is obtained. In the USA, the majority of retina specialists are using bevacizumab as the first-line drug (American Society of Retina Specialists: Preferences and Trends Survey 2018.). Thus, due to its worldwide massive use, mainly related to the significantly lower price, bevacizumab is considered to be the most cost-effective drug for neovascular AMD (Elshout et al., 2018, Low et al., 2019, Stanca et al., 2019).

The introduction of additional fluid into the vitreous cavity by intravitreal therapy can cause an immediate rise in intraocular pressure (IOP). This transient, short-term IOP elevation (lasting up to 30 minutes) after intravitreal anti-VEGF therapy has been firstly reported by Falkenstein et al., in 2007. To further evaluate the impact on the IOP, a prospective, nonrandomized study was initiated over a period of 1 year in patients treated with multiple intravitreal injections of bevacizumab (Avastin®) or aflibercept (Eylea®).

Some patients with CNVs are either primary non-responders or become refractory in time to different anti-VEGF agents. Lux et al., found that 45% of the patients with CNVs due to AMD were non-responders to conventional therapy and the predictor of treatment failure was the extent of lesions, significantly larger in the non-responders

group. A possible explanation for the decreasing response to treatment in time can be related to monthly bevacizumab administration.

This might decrease the bioefficacy of the drug, a phenomenon known as tachyphylaxis (Schaal et al., 2008). As increasing the dose or reducing the interval between administrations failed to overcome tachyphylaxis, one of the newest strategies in refractory CNV is to switch to another anti-VEGF drug (Amoaku et al., 2015).

Bakall et al. reported the potential beneficial impact of aflibercept in AMD patients with CNVs refractory to bevacizumab or ranibizumab. Aflibercept is one of the newest approved anti-VEGF drugs, a powerful recombinant fusion protein that binds to all isoforms of VEGF-A, VEGF-B, and placenta growth factor (PGF). Aflibercept has the highest affinity for VEGF-A as compared to all other anti-VEGF (Hsia et al., 2015) and proved higher anatomic efficacy than bevacizumab or ranibizumab (Schmidt-Erfurth et al., 2014). Apart from inhibiting angiogenesis, anti-VEGF drugs reduce vascular leakage and edema leading to a subsequent reduction in the macular thickness (Wang et al., 2013). One published manuscript aimed to evaluate the short-term morphological and functional changes when bevacizumab was switched to aflibercept in cases transformed into refractory CNVs.

In two other published studies, the aim was to assess the anatomical and functional results after intravitreal bevacizumab administration in choroidal neovascularization secondary to pathologic myopia and choroidal neovascularization due to angioid streaks as they represent significant causes of retinal blindness in adults.

2.2.3. Material and methods

In order to review the **influence of unilateral intravitreal bevacizumab injection on the incidence of symptomatic choroidal neovascularization in the fellow eye in patients with neovascular age-related macular degeneration**, the authors performed an extensive literature search in the Medline electronic database, using the PubMed interface. The keyword combinations used were ‘intravitreal bevacizumab injections’, ‘choroidal neovascularization’, and, in turn, each of the following: ‘fellow eye’, ‘neovascular age-related macular degeneration, “incidence”. We included articles in English, published in the last 10 years. After filters were applied (case report, classical article, guideline, journal article, meta-analysis, observational study, review, systematic review) a consistent number of references resulted. Among these, 35 references were cited in this review. My personal experience in the field is enclosed in the review, as a result of a retrospective, non-comparative analysis of a consecutive group of patients with unilateral neovascular AMD at baseline, treated with intravitreal bevacizumab exclusively. Informed consent was obtained from each patient. The patients received 3 monthly intravitreal injections of 1.25 mg/0.05 ml bevacizumab followed by additional injections on a treat and extend basis, as a part of the current treatment protocol in neovascular AMD at ‘Retina Center’ Eye Clinic in Iasi. The patients were followed up for 36 months.

The **incidence of ocular hypertension after intravitreal injection of anti-VEGF agents** in the treatment of neovascular AMD was evaluated in a prospective, nonrandomized study. 58 eyes diagnosed with neovascular age-related macular degeneration and receiving „Pro Re Nata” (PRN) intravitreal treatment with anti-VEGF agents (bevacizumab or aflibercept) were included in this study. The follow-up period was 1 year. Inclusion criteria consisted of age between 65 and 85 years, initial IOP < 21 mmHg, ability to understand and sign the consent form, and ability to follow the

scheduled visit protocol. The exclusion criteria consisted in: open-angle or angle-closure glaucoma, suspected glaucoma (IOP > 21 mmHg and/ or cup to disc ratio > 0.5), currently receiving a systemic beta-blocker, previously receiving intravitreal injection of any medication (steroid, ganciclovir, and anti-VEGF agent), current use of steroid eye drops, and any ocular surface disease precluding a reliable IOP measurement.

The follow-up protocol of patients with exudative AMD treated with anti-VEGF required strict monthly follow-up visits with complete ocular examinations, including IOP measurements. Intraocular pressure was measured by using the Goldmann applanation tonometry before the intravitreal injection, at 24 hours after the administration of the anti-VEGF agent at 1 and 4 weeks. Ocular hypertension was defined as intraocular pressure over 21 mmHg. Patients diagnosed with glaucoma or who underwent ophthalmic surgery were excluded. All the patients received a loading dose, which consisted of one monthly-administered injection, for three months.

The patients were divided into two groups according to the injection type:

• 50 (86%) patients received bevacizumab

- 36 (72%) patients received the injection at an interval of less than 8 weeks (range 4-7 weeks) after the initial loading dose

- 14 (28%) patients received the injection at an interval of more than 8 weeks (range 8-12 weeks) after the initial loading dose

• 8 (14%) patients received aflibercept (once every 2 months after the initial loading dose).

In order to evaluate the **efficacy and safety of aflibercept as a "rescue" therapy in refractory CNVs,** a series of 8 cases with refractory CNVs to bevacizumab were evaluated. The CNVs were associated with neovascular AMD in 7 out of 8 cases, and angioid streaks in 1 case. All cases benefited from a complete monthly ophthalmic evaluation, including best-corrected visual acuity (BCVA) and spectral-domain optical coherence tomography (SD-OCT).

Most of the patients included in this case series were old, with a mean age of 67.6 years (54-74 years). Each patient was initially successfully treated with repeated doses of 1.25 mg bevacizumab, the mean number of intravitreal administrations of bevacizumab before switching to aflibercept was 9.32 (7–12). In all cases, the last 3 intravitreal injections of bevacizumab, performed at a maximum 6 weeks interval, were ineffective proving the refractory status of CNVs. The lack of improvement or even worsening of the clinical features was also documented by the SD-OCT evaluation.

The efficacy and safety of **intravitreal bevacizumab in choroidal neovascularization secondary to pathologic myopia and angioid streaks** were evaluated in a prospective, interventional case study of 18 eyes and 8 eyes respectively. The secondary choroidal neovascularization was treated with 1.25mg. intravitreal bevacizumab (AVASTIN®). Intravitreal injection was repeated, if needed, at 4-6 weeks until leakage stopped.

In all cases, fluorescein angiograms and Spectral 3D OCTs were performed. Visual acuity was measured with ETDRS optotype. Cases were followed-up at least 6 months. Statistical analysis was performed using ANOVA and Wilcoxon tests.

2.2.4. Results

As in many retinal diseases, one key problem in neovascular AMD is the fellow eye involvement. Literature data largely vary on this topic. In clinical trials, fellow eye involvement was noted at 22, 24 and 36.3% of patients by 24 months (Solomon et al., 2007, Brabazetto et al., 2010, Parikh et al., 2019), regardless of the medication. A recent real-life evaluation reported a 32% fellow eye involvement rate at two years (Fasler et al., 2020). Severe macular changes in the affected eye (larger membranes, more intraretinal fluid) in addition to increased age, female sex, and genetic disposition correlate to a higher risk of second eye involvement (Rasmussen et al., 2017). Optical coherence tomography angiography (OCTA) evaluation suggests that the presence of subclinical CNV in the fellow eye is associated with an increased risk of exudation (de Oliveira et al., 2018, Yanagi et al., 2018).

Another key problem is the influence of anti-VEGF treatment in one eye on the fellow eye that has no clinical signs of neovascularization at baseline. Post hoc analysis of some major randomized, double-masked, active-controlled, multicenter clinical trials could not reveal any consistent influence of intravitreal ranibizumab or aflibercept injections on fellow eye conversion rates. When studying bevacizumab, also, no difference was noted in the first study year (7.2% of patients treated with bevacizumab vs. 7.9% of patients treated with ranibizumab). After 2 years, a difference was noted, although not statistically significant (16.6% of patients treated with bevacizumab vs. 20.6% of patients treated with ranibizumab (Maguire et al., 2013).

Fellow eye effects due to systemic exposure of bevacizumab have been reported, in real life, from its very early use in many retinal diseases including proliferative diabetic retinopathy, diabetic macular edema, type 2 idiopathic macular telangiectasia, uveitic cystoid macular edema and retinopathy of prematurity (Avery et al., 2006, Garcia-Quintanilla et al., 2019, Avery et al., 2006). A recent publication reports a greater therapeutic effect of unilateral bevacizumab in the fellow eye when compared with the other two anti-VEGF agents (Malbin et al., 2019). In the particular case of neovascular AMD there are many reports on clinically significant improvements of the untreated fellow eye after unilateral injections of anti-VEGF agents. They concern visual acuity, fluorescein angiography, and/or central macular thickness as measured by spectral-domain optical coherence tomography (SD-OCT) (Isildak et al., 2018). In a prospective, non-randomized trial, patients with unilateral neovascular AMD were treated with intravitreal injection of ranibizumab or bevacizumab and fellow-eye changes of central retinal artery equivalent (CRAE) and central retinal vein equivalent were measured postoperatively using image analysis software. A significant transient narrowing effect on the CRAE was noted in the fellow non-treated eyes of the bevacizumab group only three days after injection (Peyman et al., 2018). This reaction is supposed to be the consequence of bevacizumab interference with nitric oxide production from vascular endothelial cells.

After the study approval by the Ethics Committee of ‘Retina Center’ Eye Clinic (Iasi, Romania), the authors performed a retrospective, non-comparative analysis on a consecutive group of patients with unilateral neovascular AMD at baseline, treated with intravitreal bevacizumab exclusively. After reviewing data from 255 patients followed up for 36 months, it was found that symptomatic CNV developed in 5 fellow eyes at 12 months (3.08%), 19 fellow eyes at 24 months (10.55%), and 29 fellow eyes at 36 months (17.90%).

The main reason for the extensive worldwide **"off-label" usage of intravitreal bevacizumab** was, and still is, a financial one. Bevacizumab has similar efficacy to other anti-VEGF agents but costs, for example, 40 times less than approved ranibizumab. An increasing number of patients suffering from wet AMD, diffuse diabetic macular edema, or macular edema secondary to venous occlusions asked for reimbursement of repeatedly, sometimes monthly, treatments with approved anti-VEGF drugs thus creating a significant burden on both health providers and insurance companies. In developing countries, patients' access to such approved drugs is still very limited as the insurance system cannot fully reimburse them. In these circumstances, it is understandable why both the ophthalmologist and the patient agree, in most cases, for "off-label" bevacizumab usage as the only affordable alternative for preserving vision. The massive intravitreal use of bevacizumab for different indications of anti-VEGF therapy in ophthalmology has generated an impressive international experience and the literature in the latest years is consistent in reports concerning this topic. Although the clinical impression was of similar efficacy to approved drugs there were questions related to safety as some reports included side effects possibly related to the non-intravitreal formulation or thromboembolic events related to systemic appearance (Beer et al., 2006, Zehetner et al., 2013). These suspicions constrained the international scientific communities to study the non-inferiority of bevacizumab in comparative, prospective and randomized clinical trials. The CATT-trial (Comparison of Age-related Macular Degeneration Treatments Trials) and IVAN-study (Inhibition of VEGF in Age-related Choroidal Neovascularization study), performed in the USA, respectively England and Northern Ireland, confirmed, at both 1 and 2 years follow-up, the similar efficacy of ranibizumab and bevacizumab. Comparative results were also obtained in morphology. The side-effects analysis suggested in CATT-trial a significant 1.3 greater risk in bevacizumab treated group for ocular and systemic events in both 1 and 2 years follow-up. There was no significant difference instead in death or thromboembolic events. In IVAN-study, in the contrary, there was no difference in side effects. Up to now, the investigators are still looking for an explanation concerning this difference in results. Financial analysis revealed a clear disadvantage for much more expensive ranibizumab.

Regarding the study evaluating the **incidence of ocular hypertension after intravitreal injection of anti-VEGF agents in the treatment of neovascular AMD**, fifty-eight eyes of 58 patients diagnosed with neovascular age-related macular degeneration were analyzed. The mean age was 69 years (ranging from 65–85 years of age) and most of the patients were women. The study included 36 phakic eyes (62%) and 22 pseudophakic eyes (38%).

Most patients received intravitreal bevacizumab injection (86%) and the rest were treated with intravitreal aflibercept (14%). All the patients were treated with the standard dose of Avastin or Eylea administered intravitreally. Most patients, 72%, received the bevacizumab injection at an interval of less than 8 weeks after the initial loading dose. The patients receiving aflibercept were treated according to the recommended protocol (once at every two months after the initial loading dose) (Figure 2.1).

The patients received an average of 7.54 intravitreal injections during the study period, with limits between 3 and 10 injections. The only complications encountered post injections were discreet corneal edema, conjunctival congestion, and subconjunctival hemorrhage.

IOP was monitored for all patients before treatment and at all follow-up visits. Sustained IOP elevation was defined as an IOP of 21 mmHg or greater, combined with a rise of 6 mmHg or more lasting for 30 days or more, compared to baseline. The average

IOP rose from 15.3 mmHg (range 8-15 mmHg) before the initiation of injections to 19.8 mmHg (range 16-28 mmHg) after the treatment.

No significant differences were found in IOP elevations between the two groups depending on the anti-VEGF agent used (bevacizumab or aflibercept) (Figure 2.2), although bevacizumab seemed to induce a slightly higher IOP level than aflibercept.

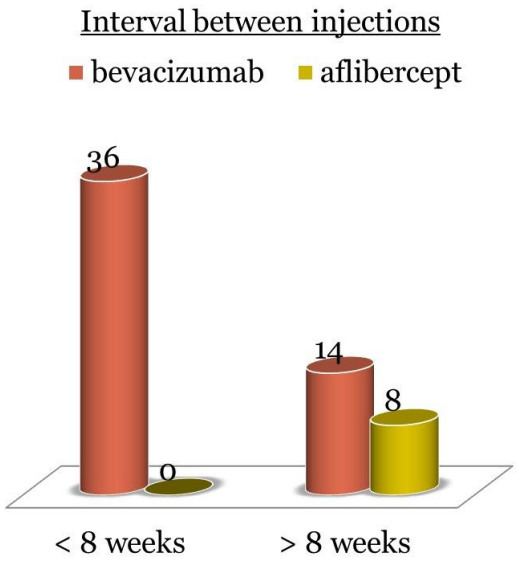


Figure 2.1 - Frequency of treatment after the administration of the loading dose of bevacizumab/ aflibercept.

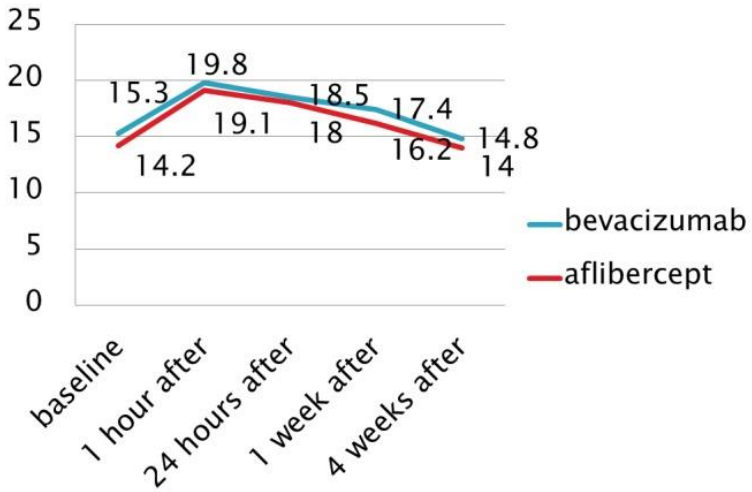


Figure 2.2 - Variation of mean IOP values after the intravitreal injection.

The raised intraocular pressure was related to an increased frequency of treatment. Increased levels of the IOP were observed in patients who received more than six injections and those in whom the frequency of the treatment was of less than eight weeks. The interval between injections was the most remarkable risk factor for the IOP elevation identified in this study. The IOP elevation was greater when the average interval between the injections was of less than eight weeks compared to the average intervals of eight weeks or more.

At 1 year follow up, an average difference of 2.1 mmHg compared to baseline was registered in the cases that have received more than 6 intravitreal injections. By comparison, in the cases treated with a reduced number of doses of intravitreal anti-VEGF agent, the difference from baseline was 0,9 mmHg.

None of the patients had to undergo glaucoma surgery or discontinue their participation in the study following uncontrolled IOP. Four patients needed topical hypotensive treatment during the follow-up period due to the maintenance of IOP at levels higher than 25mmHg.

Regarding the **efficacy of aflibercept in refractory choroidal neovascularization**, a total of 5 out of 8 cases (62.5%) showed an anatomical improvement after switching from bevacizumab to 2.0mg aflibercept intravitreal injection. The anatomical recovery paralleled all the 3 monthly administrations. In particular, SD-OCT proved as the main monitoring tool, providing the finest evaluation of RPE configuration and subretinal fluid and intraretinal abnormalities evolution. A brief description of these cases is presented below, along with the OCT scans.

Case 1

A 65-year-old male patient who successfully underwent 6 previous administrations of 1.25 mg bevacizumab for subfoveal CNV due to neovascular AMD, showed worsening of both BCVA and clinical aspects during the last 3 injections with persistent subretinal fluid (Figure 2.3).

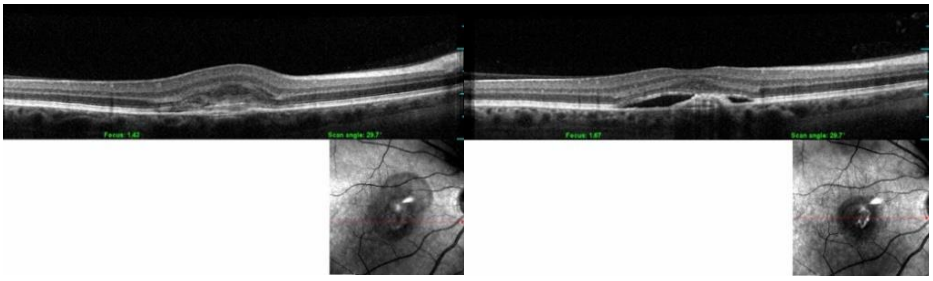


Figure 2.3 - Worsening of anatomical condition during the last 3 bevacizumab injections.

After the first injection of aflibercept, a mild anatomical improvement was noticed, which continued throughout the treatment (Figure 2.4). The anatomical improvement was not paralleled by the BCVA improvement.

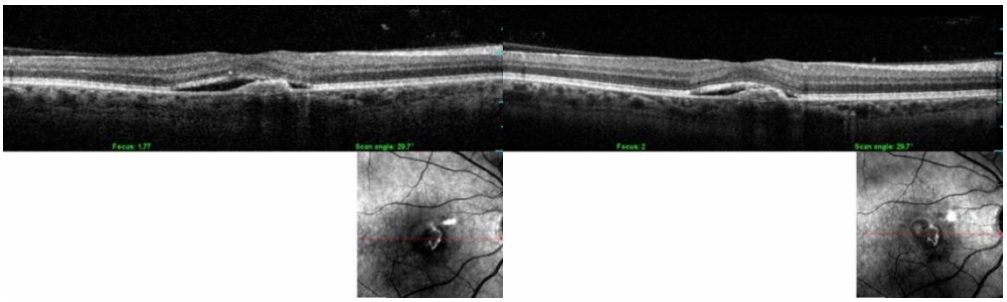


Figure 2.4 - After the 1st and 3rd aflibercept injection – continuous reduction in both RPE elevation and subretinal fluid.

Case 2

A 73-year-old male patient with neovascular AMD, received a total of 6 injections of intravitreal bevacizumab, with a lack of improvement during the last 3 injections (Figure 2.5).

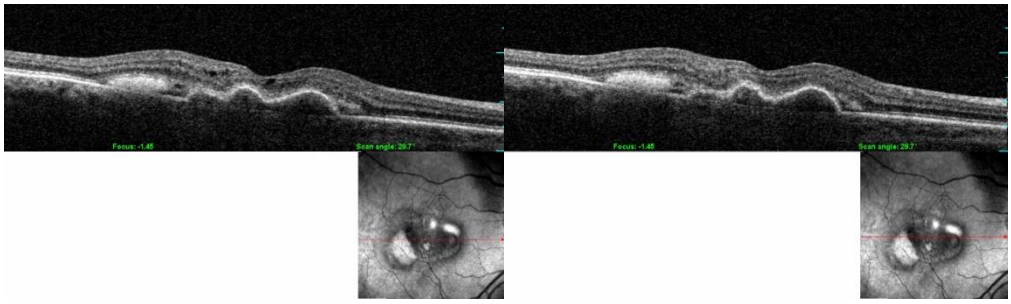


Figure 2.5 - No anatomical improvement during the last 3 injections of bevacizumab.

After switching to aflibercept, the anatomical aspect significantly improved immediately after the first injection. After the 3rd injection of aflibercept, the PED disappeared but a large intraretinal degenerative cyst was still present (Figure 2.6). No BCVA improvement was noticed.

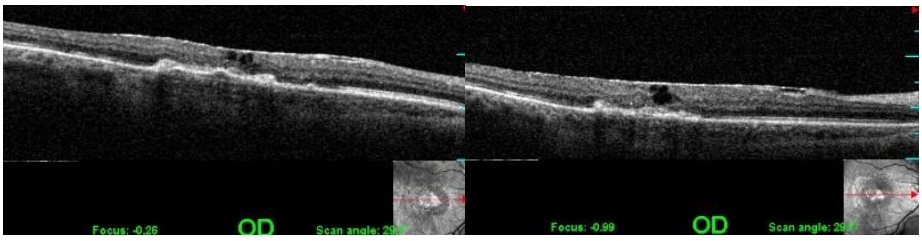


Figure 2.6 - Anatomical improvement after the 1st and 3rd aflibercept injections: PED disappearance and intraretinal degenerative cyst persistence.

Case 3

A 54-year-old male patient received a total of 5 bevacizumab injections for juxtafoveal CNV due to angioid streaks. During the last 3 administrations of bevacizumab, the OCT examination showed a further thickening of the macula and an increase in the cystoid spaces (Figure 2.7).

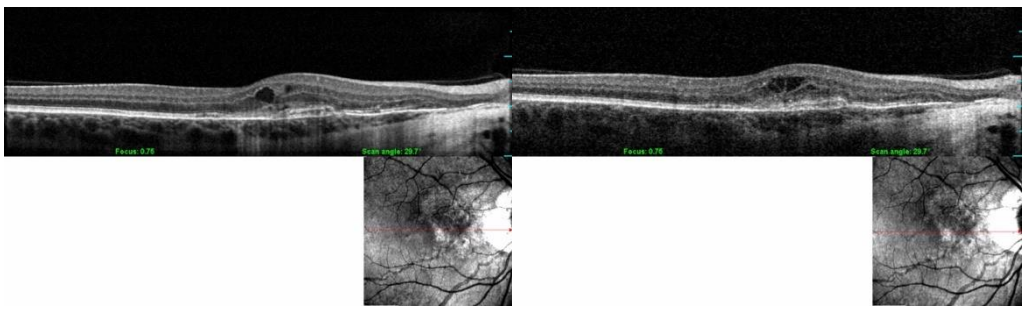


Figure 2.7 - CNV due to angioid streaks worsening during the last 3 intravitreal bevacizumab administrations.

The aflibercept therapy in this case greatly improved both the macular architecture and the BCVA. The OCT cross-section after the 3rd aflibercept injection revealed the disappearance of cystoid spaces and the reduction of the macular thickness (Figure 2.8).

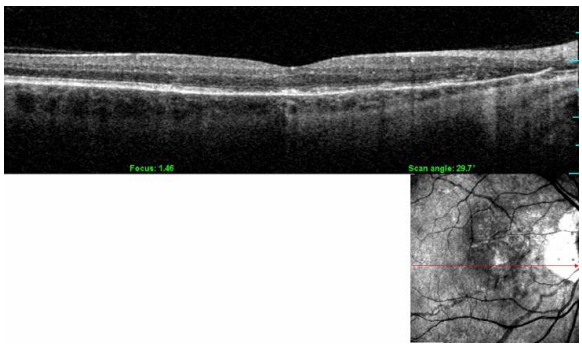


Figure 2.8 - Improved macular scan after 3 aflibercept injections.

Case 4

A 69-year-old female patient underwent 9 previous bevacizumab intravitreal injections for CNV, due to neovascular AMD. After a significant clinical improvement, a large new unresponsive PED developed despite the additional treatment (Figure 2.9).

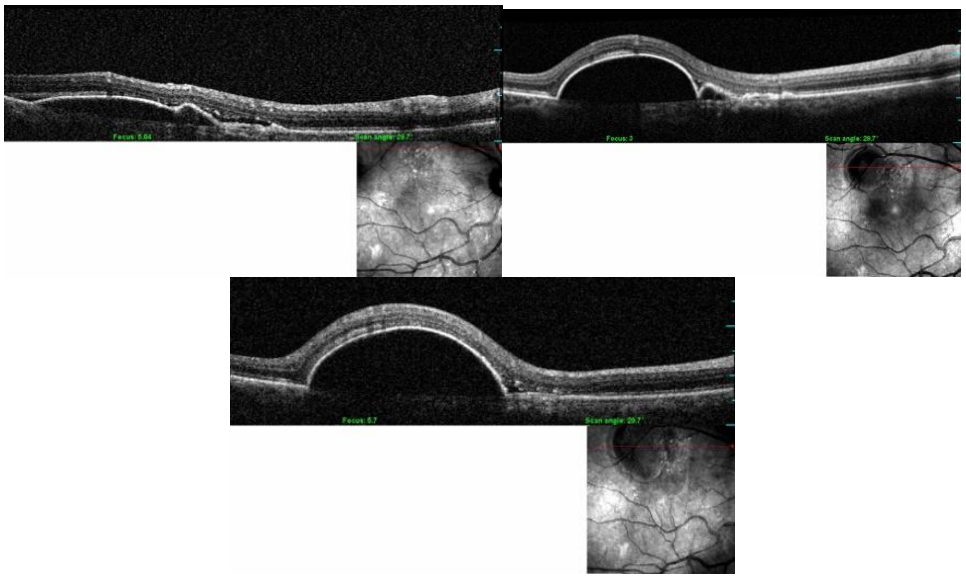


Figure 2.9 - OCT scans and the SLO fundus images show a significant worsening of the macular lesions despite the 3 monthly bevacizumab intravitreal injections.

After switching to aflibercept, a slow progressive anatomical improvement was noticed in both reductions of PED height and extension (Figure 2.10). Unfortunately, only a mild improvement in BCVA was noticed.

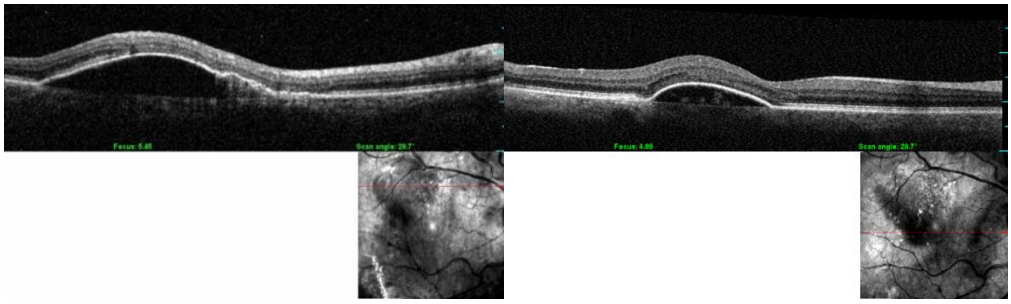


Figure 2.10 - Progressive PED reduction after the 1st and 3rd aflibercept injections.

Case 5

This case was somehow particular because of the patients' outstanding improvement after switching the anti-VEGF therapy from bevacizumab to aflibercept. The 71-year-old male patient, with a large area of serous retinal detachment due to neovascular AMD, proved quickly unresponsive after 2 intravitreal injections of 1.25mg bevacizumab. Total remission of subretinal fluid was obtained with only 2 intravitreal injections of 2mg aflibercept (Figure 2.11). Also, a mild improvement in BCVA was noticed.

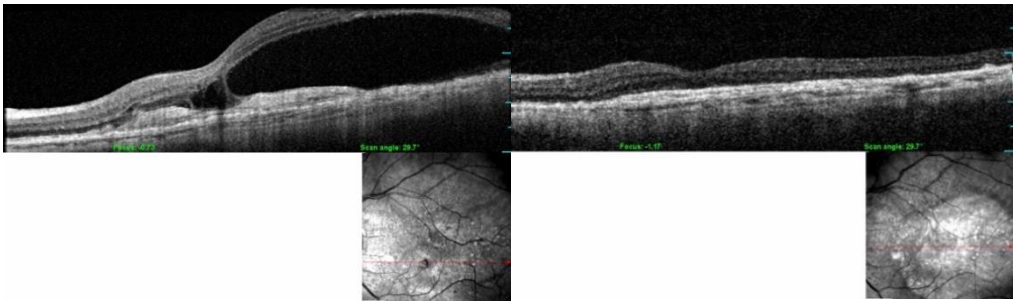


Figure 2.11 - Total remission of subretinal fluid after 2 aflibercept intravitreal injections.

Regarding the efficacy and safety of intravitreal bevacizumab in choroidal neovascularization secondary to other retinal diseases (pathologic myopia and angioid streaks), the results can be resumed as follows. The mean age of patients in the study of **intravitreal bevacizumab efficacy and safety in choroidal neovascularization secondary to pathologic myopia** was 43.86 ± 6,32 years (ranging from 24-62 years). The mean number of intravitreal injections was 2.62 ± 0,53 (ranging between 1 – 4 injections). Mean visual acuity improved in all cases. An increase of more than 15 letters was noted in 44.44 % of the cases. OCT confirmed a reduced depth of the lesion and also a reduced lesion volume after treatment. No major local or systemic side effects were noted. At 6 months follow-up the choroidal neovascularization reappeared in 5 cases (27.77%) requiring additional treatment. Eight cases with **CNV associated with angioid streaks** were evaluated between January 2007 and January 2013. The mean age of patients in the study was 52,36 ± 4,33 years (ranging from 42-64 years). The mean number of intravitreal injections was 4.64 ± 0,42 (ranging between 3 – 8 injections). Mean visual acuity improved significantly in all cases after 3 intravitreal injections with a gain of more than 15 letters in 6 out of 8 cases (75%). OCT confirmed the reduced depth of the lesion and also a reduced lesion volume after treatment. No major local or systemic

side effects were noted. At 6 months follow-up the CNV reoccurred in 5 out of 8 cases (62.5%) requiring additional treatment. 3 out of 8 cases finally lost more than 5 letters due to subretinal fibrosis.

2.2.5. Discussions

Regarding the influence of the anti-VEGF treatment on the fellow eye, these results can be explained, at least in part, by the systemic exposure and the significantly longer reduction of VEGF serum levels after intravitreal injections of bevacizumab. A consistent reduction in human serum VEGF levels has been observed after intravitreal injections of all anti-VEGF agents but is more prominent after the use of aflibercept and bevacizumab (Zehetner et al., 2013, Chakravarthy et al., 2012). Different human studies have focused on establishing the systemic half-life of anti-VEGF agents injected intravitreally (Avery et al., 2014, García-Quintanilla et al., 2019). In the case of ranibizumab, the systemic half-life was estimated at 2 h after one injection and 5.8 days after 3 injections of 0.5 mg. The serum half-life of aflibercept is estimated at 11.4 days after three-monthly intravitreal injections of 2.0 mg while bevacizumab has the longest serum half-life, around 18.7 days, after three intravitreal doses of 1.25 mg. Both aflibercept and bevacizumab manifest systemic drug accumulation between the first three doses while ranibizumab has the quickest bloodstream clearance that does not allow significant systemic accumulation. These findings are confirmed by the IVAN study, one of the most important trials of the last decade. The analysis of serum VEGF levels in patients with neovascular AMD revealed a more consistent and long-standing VEGF suppression induced by bevacizumab as compared with ranibizumab (reduction of 69% for bevacizumab and 20% for ranibizumab at 1 year, and a reduction of 78% for bevacizumab and 28% for ranibizumab at 2 years).

The results obtained on our retrospective evaluation are somehow intriguing because of lower incidence as compared with observational large-scale studies involving other anti-VEGF agents and suggest a possible interference of bevacizumab with the fellow eye conversion rate.

The **“off-label” term** is generally assigned to a drug used differently as approved whether for a different indication, a different dosage, a different way of administration or addressed to another age group (Randall et Stafford, 2008). FDA (Food and Drug Administration) in the United States and EMA (European Medicines Agency) in European Union approves the usage of new therapeutic agents only after a rigorous evaluation concerning efficacy and safety in prospective, randomized, clinical trials based on previous consistent research. The validating procedures involve considerable human and financial resources and can delay up to 7 years the usage of a new drug (Nielsen et al., 2004). Once approved, the drug can be used only according to the validated results of the research. Using an “off-label” drug is not unusual. In the United States, in the late ’90s, approximately 60% of the drugs were prescribed in this manner (James et al., 1998). More recently, the percentage dropped to 30%, most of the drugs administered in this manner being indicated in psychiatry, allergies, cardiovascular and gastrointestinal diseases (Radley et al., 2006). For example, in the Treatment Guide of Urticaria, published by Zuberbier in 2009 there is a recommendation to increase 4 times the dose of nonsedating H1 antihistaminic (nsAH1) if the symptoms persist for more than 2 weeks under 1 nsAH1 tablet per day. This recommendation, although issued by competent medical associations (EAACI - European Academy of Allergology and Clinical Immunology, GA2LEN - Global Allergy and Asthma European Network, EDF -

European Dermatology Forum, WAO-World Allergy Organization) is an « off-label » as the dose is higher than recommended in nsAH1 use approval.

From the ethical and legal point of view, the « off-label » prescription of a drug is a delicate balance between a doctor's decision to choose the best treatment option and the patient's right to be protected against an unsafe or inefficient therapy. For most Law Courts in the United States, « off-label » practice is neither illegal nor malpraxis but involves the full responsibility of the doctor in that case. More than that, an « off-label » prescription is not considered human experimentation and not blamed if the doctor, in his effort to offer a better therapy, acted according to an already used method. In case of a conflict related to the « off-label » use of a certain drug, the resolution relies on the doctor's intention: if the primary intention was represented by achieving the maximum benefit to the patient, then the « off-label » use had a therapeutic purpose. If on contrary, the primary intention was to test a hypothesis and to obtain further knowledge, the « off-label » procedure can be considered human experimentation. Moreover, financial interest will enhance the experimental intention.

In ophthalmology intravitreal injections of vancomycin, triamcinolone acetonide, intracameral injections of lidocaine or tissue plasminogen activator or even frequent subconjunctival injections of gentamicin or dexamethasone are examples of "off-label" use. If modern medicine would use drugs strictly according to recommendations the progress in medicine would be much slower. This is not excluding that the doctor should take the treatment decisions only considering the maximum benefit for the patient. More than that, it is advisable that the information concerning "off-label" usage to be documented in peer-review journals. In the United States and Great Britain, there is no law to restrain the doctor to use a drug differently than approved if there is evidence of better efficacy and safety (GMC, 2007).

Concerning the influence on the intraocular pressure, the evidence collected in recent years has demonstrated that **intravitreal injections may produce short- and/ or long-term elevations in IOP**. The possible mechanisms for a sustained IOP elevation after the intravitreal injection of anti-VEGF agents are not very well understood. Anti-VEGF agents may directly damage the trabecular meshwork, so one possible mechanism of IOP elevation is inflammation, such as drug-induced trabeculitis or uveitis (Kiddee et al., 2015). Another mechanism of anti-VEGF-induced IOP elevation is considered an underlying inflammatory mechanism or an immunological reaction that damages the aqueous humor outflow pathways. Besides that, a traumatic mechanism after frequent injections may lead to a disruption of the anterior hyaloid or zonules and allow access for high molecular weight proteins to enter the anterior chamber, resulting in increased IOP (Bakri et al., 2008).

The IOP spike that occurs after the intravitreal injection of anti-VEGF agents is usually transient, with IOP returning to a safer range (< 25–30 mmHg) within 1 hour, without topical hypotensive medications, in most patients (Avery et al., 2006). A prolonged elevation was found in 4 out of 58 patients treated with recurrent intravitreal Eylea or Avastin who needed topical hypotensive treatment during the follow-up period. None of the patients who suffered from IOP elevations had a history of glaucoma or ocular hypertension. Some authors reported an increased risk of IOP elevation in patients receiving >29 injections compared to patients with <12 injections (Grismond et al., 2009). Moreover, an increased risk of IOP elevation was also found in the groups with a higher number of injections. Initial IOP elevation varied from 1 hour, 1 week, and 3 months after treatment. In 3 eyes, the IOP returned to baseline levels after 1 month, even when receiving monthly injections. A possible explanation for the low rate of sustained IOP

elevation could be that 64% of the patients in our study did not receive monthly injections, but rather, received an extended treatment regimen. The increase of the interval between injections may allow the anti-VEGF medication to be cleared from the eye (Wehrli et al., 2012).

The type of injected drug (Avastin versus Eylea) did not have a statistically remarkable influence on the difference in IOP, but a slightly higher IOP was found in eyes receiving bevacizumab. The limitations of this study include its retrospective nature and a short follow-up period, which implied a limited number of injections.

The **intravitreal administration of aflibercept** proved efficient and safe as rescue therapy in the retrospective analysis of our cases with **CNVs refractory** to 1.25 mg intravitreal bevacizumab switched to 2.0 mg intravitreal aflibercept. Five out of six cases (62.5%) had a clinical and anatomical improvement confirmed by the SD-OCT findings. The other 3 cases rather showed a clinical stabilization during the aflibercept treatment with no additional BCVA loss.

Only 3 out of 5 improved cases (60%) also had a visual benefit, recovering a mean of one line of visual acuity. The other 2 patients might still have a chance for visual improvement in time, as BCVA does not always immediately parallel anatomical amelioration. On the other hand, Wickremasinghe et al. noticed that during the anti-VEGF therapy, BCVA could decrease in the absence of fluid on the OCT scans. However, if present, intra-retinal fluid is associated with poorer visual acuity. Many studies have already suggested the positive effect of aflibercept therapy in patients with neovascular AMD and CNVs resistant to either bevacizumab or ranibizumab (Sellam et al., 2014, Cho et al., 2013). In a retrospective analysis published in Retina, Kumar et al. reported that eyes with neovascular AMD resistant to intravitreal ranibizumab or bevacizumab improved under intravitreal aflibercept therapy. The anatomical improvement was confirmed by the reduction of subretinal fluid, intraretinal fluid, and subfoveal PED on OCT images. However, as it was also noticed in our series, regarding the visual function, Kumar et al., pointed out that there was no significant improvement in BCVA after 3 consecutive aflibercept administrations, and that after additional aflibercept injections there was a statistically significant but minor visual improvement. In such cases of refractory CNVs, most authors agreed that the visual acuity rather stabilizes and rarely improves.

Hsia et al. reported in a smaller number of cases, an anatomical improvement in all 5 refractory cases switched to aflibercept and an increase of BCVA in 4 out of 5 eyes. Of course, these results are limited to a small number of cases.

Aflibercept intravitreal injections were well tolerated. In our study, no ocular or systemic complications were noted during and after the intravitreal injection of standard dosage.

The limitations of this study are related to the retrospective design but mostly to the small number of cases evaluated. As already suggested, the OCT proved as a quick non-invasive, safe, and very effective tool to monitor the response to anti-VEGF intravitreal therapy.

2.2.6. Conclusions

If **unilateral intravitreal injections of bevacizumab are influencing the conversion rate of the fellow eye in patients with unilateral neovascular AMD** at baseline is still unknown and there is a lack of literature on this topic. Until now, the more substantial systemic exposure of bevacizumab after intravitreal injection as compared

with ranibizumab and aflibercept has raised challenging discussions concerning only an increased risk of systemic adverse events (especially cerebrovascular accidents) and the interference with VEGF-dependent physiological processes (especially in newborn babies). It also looks very plausible that long-term unilateral intravitreal injections of bevacizumab in neovascular AMD could prevent the appearance of symptomatic neovascularization in the fellow eye as suggested by the results of our retrospective 36-months evaluation. Whether or not this intriguing effect is significant needs to be confirmed by larger, comparative studies.

"Off-label" prescriptions, and in particular the extensive usage of AVASTIN® in ophthalmology for more than 10 years, including Romania, raised numerous controversies related to legitimacy and ethics. Although most of the concerns related to lower efficacy and higher complications rate have been invalidated by prospective clinical trials, the status of this therapy for ophthalmic use has not changed. Therefore, the use of this drug, like all "off-label" prescriptions, should be performed with caution, only by reputed and well-documented specialists. The informed consent must contain and emphasize the "off-label" status of the proposed therapy and also must offer detailed information about alternatives, risks, and benefices. In ophthalmology, in many situations, the choice for bevacizumab for so many years was the result of the balance between the risk of irreversible losing vision and the lack of reimbursement for more expensive approved drugs the patient could not fully sustain.

Most eyes from the study of **ocular hypertension after intravitreal injection of anti-VEGF agents** achieved normalization of IOP within 24 hours without the need for any immediate intervention. Close monitoring of IOP is mandatory, especially in those patients receiving frequent injections, in order to prevent further neural damage, in addition to the underlying retinal disease.

The data collected from the study on **refractory CNVs** suggest that most of the patients resistant to intravitreal bevacizumab may benefit from the therapeutic change to intravitreal aflibercept. Unfortunately, the anatomical improvement, spectacular in at least 2 of our cases, was not always paralleled by a significant increase in BCVA. In our experience, aflibercept proved to be an effective rescue therapy in such cases, with good efficacy and safety. These results can be, at least partially, explained by the more complex biochemical interactions of aflibercept. A larger number of patients and further studies are necessary to assess this new therapeutic indication, the long-term efficacy and safety in such cases.

The results obtained in the prospective studies confirm the **efficacy and safety of intravitreal bevacizumab in controlling the CNVs due to pathologic myopia and angioid streaks.** More than 40% of the cases with pathologic myopia regained at least 3 lines in ETDRS chart but a recurrence was noted in 27.77% of the cases at 6 months. High recurrence rate and quick lesion progression to subretinal fibrosis in angioid streaks might be responsible for long-term poor functional results in this type of CNVs.

SECTION III. FUTURE DIRECTIONS IN THE PROFESSIONAL, ACADEMIC, AND SCIENTIFIC ACTIVITY

III.1. DEVELOPMENTS IN THE ACADEMIC AND PROFESSIONAL ACTIVITY

While professional recognition usually arrives in time and is a solid benchmark, the appreciation of the academic activity is more difficult to achieve and is a result of multiple factors. In my opinion, the most important tasks that I have followed during the years and intend to improve can be summarized as follows.

- To continuously increase professional knowledge and skills and also didactic know-how through constant participation in training programs, national and international courses, conferences, and congresses;
- To actively implicate in such activities, not only as an organizer or passive participant but as an active speaker presenting personal results and opinions;
- To continuously improve all teaching programs for students, residents in ophthalmology, and young ophthalmologists according to the latest achievements in the field and with the national and international guidelines and protocols;
- To continue elaborating teaching materials in both digital and printed forms;
- To improve the evaluation methods through multistage case discussions, based on selected bibliography and relevant iconography;
- To stimulate the multidisciplinary approach with active participation in congresses, conferences, courses, and webinars;
- To stimulate young ophthalmologists' participation in local and national conferences;
- To stimulate a positive attitude in the Discipline, based on respect and equal opportunity;

III.2. DEVELOPMENTS IN THE SCIENTIFIC ACTIVITY

At this particular moment, when the habilitation thesis is elaborated, some of the future research directions that are presented below are already crystallized and need to be concluded before publication.

III.2.1. Current trends in targeting the oxidative stress in glaucoma

Glaucoma is a progressive optic neuropathy characterized by retinal ganglion cells (RGC) degeneration and visual field loss. Glaucoma is considered to be the leading cause of blindness in industrialized countries. Intraocular pressure (IOP) is the only modifiable risk factor that can slow the progression of the disease and lowering the IOP is the only proven intervention for the preservation of vision. Even if effective medical and surgical therapies exist, progressive visual loss is still a prevalent symptom in patients with glaucoma. The specific mechanisms that determine the failure of the trabecular meshwork (TM) to maintain normal levels of aqueous outflow are incompletely understood. In the TM from glaucoma donors, there were found pro-inflammatory markers, which are induced by activation of a stress response (Stowell et al., 2017).

There is growing evidence that oxidative stress (OS) contributes to the progression of glaucoma. Some authors consider that ganglion cell apoptosis observed in glaucoma is caused by a special type of ischemia (Tezel et al., 2006). The role of oxidative damage in glaucoma can be explained by the production of reactive oxygen species (ROS) secondary to a defect in the mitochondrial complex in the TM. The

nervous system is rich in lipids and the nerve cells, including the RGC, are sensitive to oxidative damage. The high metabolic rate, the oxygen degradation and the synthesis of adenosine triphosphate (ATP) are increased in these cells, while the cellular regeneration rate is restricted. Even if less is known about the pathogenesis of glaucomatous optic neuropathy, the risk factors for arteriosclerosis play a role in increasing the IOP. Insufficient autoregulation increases the chance of unstable ocular perfusion and an unstable oxygen supply, which leads to oxidative stress. The concentration of superoxide in the optic nerve head increases and the astrocytes are activated. The nitric oxide (NO) molecules are produced in excess and fuse with oxygen in the axons, resulting peroxynitrat. This compound diffuses towards the retina and induces apoptosis of the RGC.

The causes of RGC degeneration result from primary and secondary insults. Indirect damage can be done by multiple mechanisms, which include oxidative stress-induced vascular alterations and oxidative injury to the TM. These alterations lead to impaired autoregulation of blood flow to the optic nerve, impaired aqueous humor outflow, and elevated IOP. OS may cause indirect damage to RGC, mediated by abnormal immune responses and glial cell dysfunction (Chrysostomou et al., 2013). The TM of patients with primary open-angle glaucoma is characterized by specific morphologic and biochemical changes, which lead to increased outflow resistance. OS plays an important role in the pathogenesis of glaucoma. In patients with primary open-angle glaucoma, the antioxidative capacity in the aqueous humor is reduced compared to nonglaucomatous eyes and the oxidative damage in the TM is correlated with visual field defects (Sacca et al., 2007). OS can induce apoptosis, extracellular matrix production and accelerated senescence. These studies demonstrate the role of oxidative damage in the pathogenesis of glaucoma. The vascular theory indicates that vessel dysregulation plays an important role in the pathogenesis of glaucoma. Vascular dysregulation leads to ischemia and reperfusion, thereby inducing oxidative damage. The functional alterations in the TM trigger the glaucomatous pathogenic cascade, due to a progressive accumulation of oxidative damage. Oxidative damage to the deoxyribonucleic acid (DNA) of the TM has been proved to be higher in glaucomatous patients than in age-matched controls.

OS reflects an imbalance between the production of ROS and antioxidant defenses, in which oxidative processes exceed antioxidant systems. OS is a necessary mechanism for homeostasis maintenance, in which “oxidative eustress” is considered an adaptive mechanism that allows the organism to respond and adapt to different stressors (Iorio EL, 2016). The imbalance between the level of oxidation products and the antioxidant capacity of the body is one of the causes of different pathologies, with high prevalence in modern medicine. Oxidizing substances are normally formed during aerobic metabolism, the amount of which increases under certain conditions. Under such circumstances, the antioxidant physiological mechanisms that inactivate ROS, remove altered molecules and repair damaged lesions may be insufficient. Free radicals are molecular structures characterized by the presence of an unpaired electron, which gives them great instability, special reactivity and a very short survival time. OS is defined as a state in which the level of oxygen free radicals exceeds the host's endogenous antioxidant defense systems. Thus, OS results either from an overproduction of oxidants or from a depletion of defense antioxidants.

Oxygen-free radicals are: superoxide radical, hydroxyl radical, hydrogen peroxide radical (H2O2), and singlet oxygen. Although not a free radical of oxygen, NO is an important signaling molecule involved in various physiological and pathophysiological processes (Chidlow et al., 2017). The production of ROS is a physiological process that

increases in intensity with age, inflammation, infectious diseases, radiation exposure and chemical pollutants. Once formed, ROS are rapidly broken down by existing enzymatic and non-enzymatic antioxidant systems in cells. When they are overtaken by the overproduction of active species, OS occurs. Cells can tolerate moderate levels of OS and respond by increasing the synthesis of protective antioxidant enzymes. Significant OS can cause cell damage or even cell death through necrosis or apoptosis. Under the action of OS, several structures can be affected: DNA, lipids, proteins, carbohydrates. In general, the damage caused by OS can be classified into: DNA damage, oxidation of polyunsaturated fatty acids in lipids (lipid peroxidation) and oxidation of amino acids in proteins. The action of free radicals on cellular components leads to their destruction, with consequences on the architecture and function of the cell. The OS is a risk factor in various eye diseases, such as cataract, age-related macular degeneration, and glaucoma (Lawler et al., 2019). Retinal tissue is very sensitive to fluctuations in oxygen concentration. OS in glaucoma occurs in the mitochondria of the RGC and their axons. Amplified generation of ROS during glaucomatous neurodegeneration leads to protein oxidation, depletion of the cellular redox balance and cell death. In the eye, ascorbic acid has an important protective role against OS. High concentrations are found in the vitreous humor, cornea, lachrymal film and aqueous humor. Another antioxidant found in the eye, reduced GSH represents a major defense mechanism against toxic agents. High concentrations of GSH are found in the aqueous humor and in the TM. The GSH system protects the ocular tissues from the low concentrations of H2O2, while the superoxide dismutase–catalase system protects from elevated H2O2 concentrations. In vivo, studies in humans showed that IOP increase and visual field damage are related to the amount of oxidative DNA alteration. Changes in the TM are also correlated with the severity of optic nerve damage.

Molecular modifications are considered a risk factor for several diseases, but mainly for the neurodegenerative ones, such as Alzheimer, Parkinson's disease and glaucoma. Glial activation and neurodegenerative insults initiate an immune response, in order to restore tissue homeostasis and to facilitate tissue healing. OS that appears in the pathogenesis of glaucoma, along with the age-related component of OS, affects the physiological equilibrium. Macroglial cells, including retina and optic nerve astrocytes and retinal Müller cells, have an important role in glaucomatous neurodegeneration. Microglia, also a glial cell, is a specialized tissue macrophage. Chronic functional and structural alterations of these glial cells determine progressive degeneration of optic nerve axons and RGC. These glial cells have a high level of plasticity that allows them to respond quickly to any homeostatic imbalance. After transformation into an activated phenotype, glial cells may present dysfunctions in their neurosupportive abilities, increasing the vulnerability of RGC and their axons to injury. Glial cells are thought to play important roles in the initiation of an adaptive immune response in glaucoma (Tezel et al., 2007). The ability of glial cells to function as antigen-presenting cells under OS facilitates their communication with T cells. Thus, an immune response is initiated, which targets T cells to the injury site. Understanding the glial responses and functional alterations at the molecular level may offer innovative treatment options, by selectively inhibiting the glial activation response that has neurodestructive consequences.

Different studies support the roles of the immune system in glaucoma as beneficial or destructive. An immune response can be beneficial and necessary to restore tissue homeostasis and promote tissue healing, without causing an autoimmune disease. Based on the activated state of glial cells, along with the production of high levels of ROS, we can state that OS is a factor that drives a resting immune system over the threshold of antigen-specific activation (Tezel et al., 2014).

Most biological processes in living cells are based on oxidation-reduction (redox) reactions. The redox reactions aim to preserve the homeostasis of the whole organism, counteracting endogenous and exogenous ROS sources. The proper functioning of such system is fundamental for redox regulation processes. An inadequate stress response, oxidative distress, may occur and this is capable of the improper oxidation of target molecules. Antioxidants are a heterogeneous class of substances that inhibit or delay the oxidation of specific molecules, through the directly scavenging of free radicals. The antioxidants from endogenous sources, which have direct action in ROS-scavenging include amino acids, small peptides, proteins, fat derivatives and metabolism end-products. Among the small peptides, glutathione (GSH) is the most important, as it modulates cellular redox metabolism. It is a powerful scavenger against various ROS. In glaucomatous patients the GSH serum levels are lower compared to the healthy ones, supporting the fact that there is a reduced antioxidant response in these patients (Gherghel et al., 2005). Within the aqueous humor, measurable levels of free radicals are detectable, as well as a variety of antioxidant defenses. Products resulting from oxidation, which are tissue and serum markers, consist of malonyl dialdehyde (MDA), advanced lipid oxidation endproducts for lipids, and advanced oxidation protein products for proteins. In patients with glaucoma, lipid peroxidation products were found in higher concentrations in the aqueous humor and TM, compared with normal controls (Ghanem et al., 2010). An increase in MDA levels was observed in samples of lens capsule from patients with PEX syndrome. Increased MDA levels were also found in the vitreous and retina in rats with elevated IOP. Enzymatic antioxidant proteins mainly include the SOD and the peroxidases (catalase). Factors such as pro-inflammatory cytokines and prostaglandins, ROS and NO alter the activity of the antioxidant enzymes. Therefore, low concentrations of SOD are associated with OS conditions. The three known types of SOD are classified by their metal cofactor. SOD1, Cu/Zn-SOD or cytosolic SOD, plays an important role in cell survival and growth. SOD2, Mn-SOD or mitochondrial SOD, protects the cells by ROS generated by hyperoxia. SOD3, extracellular or secretory Cu-Zn-SOD, plays a protective role from the harmful effects of superoxide anion. In patients with glaucoma the levels of SOD are reduced. Peroxidases are a large family of enzymes which catalyze the transfer of one or two electrons, using hydrogen peroxide as an electron acceptor. GPX is the main protective enzyme against lipid peroxidation.

Majsterek et al., found a decreased activity of primary antioxidant enzymes (SOD, GPX and CAT) in peripheral blood of patients with glaucoma. A decrease in serum SOD and CAT activity was also observed in patients with pseudoexfoliation glaucoma, compared to age-matched controls. The ascorbic acid concentration in the aqueous humour of patients with pseudoexfoliation syndrome was significantly lower than that found in control patients. GSH levels were also decreased in pseudoexfoliation lens epithelial cells compared with non-pseudoexfoliation syndrome controls.

The only modifiable risk factor in glaucoma is intraocular pressure. But despite the effective treatment of IOP, the progression of visual field loss is prevalent in glaucomatous patients. However, neuroprotection and interventions that target the molecular mechanisms causing RGC death may enhance their survival independent of IOP control, preventing cell death after a pathological insult. Currently, an established treatment for glaucoma that involves targeting OS does not exist, but preclinical data indicate that suppression of OS may be a promising strategy.

Coenzyme Q10 (CoQ10) is a mitochondrial-targeted antioxidant that seems to have neuroprotective activity in neurodegenerative diseases and cerebral ischemia. CoQ10 associated with vitamin E administration in patients with glaucoma has a

beneficial effect on the retinal and cortical function (pattern electroretinogram and visual evoked potential improvement) (Parisi et al., 2014).

Resveratrol, a naturally occurring polyphenol found in berries, nuts, and red wine, can enhance stress resistance. Chronic treatment with resveratrol decreased the accumulation of fluorescent pigments, carbonylated proteins, and the expression of the cellular senescence marker, that are induced by OS. Resveratrol also demonstrated a significant antiapoptotic effect after acute oxidative injury.

Flavonoids are natural polyphenols that are known for their neuroprotective and antioxidative properties. Gingko Biloba contains multiple flavonoid compounds and it was shown that it can increase central and peripheral blood flow, reduce vasospasms and inhibit apoptosis. The study by Park et al., showed that ginkgo biloba improved the peripapillary blood flow in patients with normal-tension glaucoma.

Lutein and zeaxanthin are carotenoids that are found in high concentrations at the macular level. A cross-sectional study correlated dietary intake to the incidence of developing glaucoma. The results showed that patients with higher intakes of foods rich in vitamin A, C, and carotenoids had a reduced incidence of developing glaucoma (Garcia-Medina et al., 2014). Vitamin E has neurohormone-like activities and regulatory mechanisms, independent of being an antioxidant. Its serum levels increase in conjunction with the severity of glaucoma. Increased energy availability has been shown to improve vascular perfusion, reduce neuroinflammation, and enhance the resistance of RGC to mechanical and ischemic stress.

Valproic acid (VPA) is a short-chain fatty acid that is used for the treatment of epilepsy. Recently, some authors reported that VPA prevents glaucoma-like retinal degeneration in mouse models, by inhibition of the OS in RGC (Kimura et al., 2014).

Candesartan is an angiotensin II receptor antagonist that is used in the treatment of hypertension. It modulates the renin-angiotensin system, and it plays a major role in the cardiovascular system. The renin-angiotensin system is involved in oxidative stress-induced RGC death. Suppression of the renin-angiotensin system by candesartan led to RGC protection and preservation of visual function in mice (Semba et al., 2014).

Brimonidine is a α 2-adrenergic receptor agonist that is used in glaucoma patients to lower IOP. Besides the lowering IOP effect, brimonidine increases cultured RGC survival from OS damage.

Nicotinamide is a water soluble form of vitamin B3 and is the precursor of nicotinamide adenine dinucleotide (NAD), which is a coenzyme in several cellular processes. Aging causes a decrease in NAD levels leading to mitochondrial dysfunction, leaving RGC more prone to apoptosis. Animal models show that nicotinamide prevents RGC death during IOP elevation (Tribble et al., 2021).

Currently, the established treatment for glaucoma does not involve targeting OS. Preliminary data indicate that suppression of OS is a promising strategy for treating glaucoma. The delivery of antioxidants as a treatment for RGC has specific challenges. The usual delivery approaches (oral, periocular, intraocular) can limit the pharmaceutical action of antioxidants due to the presence of static barriers (sclera, retinal pigment epithelium, and multidrug resistance efflux pumps).

The final review will be soon concluded and the manuscript will be submitted for publication.

III.2.2. Highlights on genetic polymorphism associated with thromboembolic risk; possible implications in ocular and autoimmune disorders

The purpose of this research is to explore the genetic polymorphism and its association with thromboembolic retinal venous disorders, such as central/hemi-retinal vein occlusion, as well as possible correlations with other ocular findings, such as closed-angle glaucoma, but also with autoimmune general disorders.

The importance of establishing a correspondence between all above will be highlighted since they all have complex etiopathogeneses. Sometimes, when all together co-exist, they could generate effects that may be very difficult to manage. Retinal vein occlusion may coexist mostly with other factors, such as atherosclerosis, old age, diabetes mellitus, systemic hypertension, vasculitis (sarcoidosis), coagulopathies, blood hyperviscosity - due to genetic polymorphism, deficiency of thrombolytic factors and/or increase in clotting factors, migraine, and smoking, as well as with disorders that cause chronic inflammation and glaucoma. There are studies supporting that genetic polymorphism, such as the variant MTHFR A1298C, may increase the risk of developing glaucoma, especially in the heterozygote model (Ferrazzi et al., 2005). On the other hand, rheumatoid arthritis (RA) is a condition that submits patients at risk for developing cardiovascular (CV) events due to accelerated atherosclerosis, and it is an entity that causes chronic inflammation; this fact, along with the genetic background may increase the risk of CV events, regardless of the presence of traditional CV risk factors. The literature data analysis is completed with a clinical case of a female patient, previously known with rheumatoid polyarthritis, diagnosed with upper hemi-retinal vein occlusion in the one eye, and acute angle-closure glaucoma in both eyes. Extensive genetic analysis identified two mutations, PAI-1 4G allele and MTHFR A1298C mutation in heterozygosity. These results confirmed the increased risk of the patient developing venous thromboembolism or myocardial infarction, especially when associated with rheumatoid polyarthritis. The review, completed with the exemplifying clinical case, preliminary shows that it is necessary to raise awareness of all aspects of a complex medical situation, including the genetic one, in a patient's being at risk for thromboembolic episodes.

The final data will be soon revised and the manuscript will be submitted for publication.

III.2.3. A comprehensive review of differences and similarities between cutaneous and uveal melanoma and the impact on their possible association

Melanocytes are cells that produce melanin pigments in organelles called melanosomes via an enzymatic cascade that includes tyrosinase, tyrosinase-related protein-1 (TYRP1), and TYRP2/DCT (dopachrome tautomerase). Brown/black pigment eumelanin and the orange/yellow pigment pheomelanin determine skin and eye color polymorphism. Dark brown skin and eyes have a substantially higher eumelanin/pheomelanin ratio than pale skin and eyes with light-colored irises (blue, green, yellow-brown, and hazel eyes). Melanocytes are found in many regions of the human body, including the skin, eyes, cochlea, mucosal epithelia, meninges and the heart and are derived from neural crest cells. They have a well-established role and function in the skin, but not in other anatomical regions. Within organelles called melanosomes, the melanocytes are responsible of the synthesis of melanin pigments. Melanocytes in the epidermis distribute these melanin-containing melanosomes to the surrounding keratinocytes. This ensures a uniform pigmentation, determines the color of the skin, and

protects against ultraviolet radiation's detrimental effects. In the eye, melanocytes can be found in the conjunctiva and all parts of the uvea (the iris, ciliary body, and choroid). The role of melanocytes in the conjunctiva remains uncertain. Eye color is determined by the quantity and quality of melanin pigment in the iris. In contrast to the skin, the iris color remains completely unaffected by exposure to sunlight. Furthermore, the presence of melanin in uveal melanocytes has been linked to eye protection against a variety of ocular disorders that might impair vision, such as age-related macular degeneration and uveal melanoma (UM). However, the mechanism by which melanin mediates this protection remains unclear.

The skin is the most common site of melanoma development (90% of primary melanoma cases), and the neoplasm can occur in any tissue that contains melanocytes. Meanwhile, uveal melanoma is the second most common site for melanoma after cutaneous melanoma and accounts for 5% of all primary melanoma cases, representing the most frequently diagnosed primary intraocular malignant tumor in adults.

Uveal melanoma has an annual incidence of 6 people per million, whereas cutaneous melanoma (CM) has an annual incidence of 12.2 to 48.1 people per 100 000 persons. While the worldwide incidence of cutaneous melanoma has been steadily increasing in recent decades across all continents at a more rapid rate compared to any other type of cancer, the incidence of uveal melanomas has remained stable. However, there are constant variations in the occurrence of certain diseases in different regions of the world.

In both CM and UM, the incidence in European countries follows a sharp north-to-south and west-to-east decreasing curve. This gradient is directly related to the protective factor of the eye pigment present in the southern population, also respecting the high exposure to ultraviolet light in lower latitudes. Melanoma is rare in non-whites and as several studies have demonstrated, Hispanics and Asians have a lower incidence of both CM and UM while Black individuals have the lowest one.

In this research project, the authors are intending to evaluate the incidence and features of CM and UM (genetic profile, the host susceptibility factors, the environmental risk factors, the metastatic disease, the treatment, and the simultaneous disease) through extensive literature research.

III.2.4. Micropulse transscleral cyclophotocoagulation for glaucoma after penetrating keratoplasty

Penetrating keratoplasty (PK), especially when performed in complicated cases, is frequently followed by a serious and feared complication, secondary glaucoma. The incidence of glaucoma after PK varies among different studies, mostly due to difficulties in defining it. In these patients, glaucoma impacts visual function through two distinct mechanisms. Firstly, by inducing glaucomatous optic atrophy and, secondly, by reducing the transparency of the corneal graft. A gold standard for the treatment of this condition is yet to be found. Medical therapy has frequent contraindications and often becomes inefficient in time. Filtrating procedures, such as trabeculectomy or glaucoma drainage devices, are effective in reducing the intraocular pressure (IOP) but are associated with an increased rate of graft failure, due to intraoperative damage of the corneal endothelium, the toxicity of the antimetabolites and the contact between the tube and the endothelium. Graft survival seems to be particularly endangered by glaucoma drainage devices, as they produce alterations of the haemato-ocular barrier with subsequent changes in the protein content of the aqueous humor.

The use of cyclodestructive procedures, such as cryotherapy and cyclophotocoagulation, has been explored in the treatment of glaucoma post PK, usually, after other treatment options have failed, and has been associated with an increased rate of serious complications, especially hypotony and phthisis bulbi. Micropulse transscleral cyclophotocoagulation (MP-TSCPC) is a form of cyclophotocoagulation in which laser energy is delivered to the ciliary body not in a continuous, but in an intermittent fashion, in cycles consisting of short pulses of energy (the "on" periods), followed by pauses, when no energy is delivered (the "off" periods). During "on" periods, laser energy acts upon the ciliary epithelium, while during the "off" periods, adjacent tissues are allowed to cool and are thus protected by the alterations resulting from high temperatures. Consequently, MP-TSCPC has a satisfying efficiency in reducing the IOP, while also producing fewer adverse effects.

In this research, the authors will conduct a retrospective study that includes all patients with PK and secondary glaucoma treated by MP-TSCPC between January 2017 and December 2020 in the Ophthalmology Department of the Central University Emergency Hospital "Dr. Carol Davila" in Bucharest, and with a minimum follow-up interval of 12 months, which is the primary time point. Follow-up visits were scheduled at 1, 3, 6, 9, and 12 months after the intervention. The study is following the principles of the Declaration of Helsinki (2013) and has been approved by the Institutional Review Board of the hospital. Patient data is subdivided into initial and postoperative data. Initial data refers to the preoperative baseline characteristics and consist of demographic data (age, gender), the initial condition for which the PK was performed, the pathogenic mechanism of secondary glaucoma, previous surgery, crystalline lens status, corneal graft status, the best-corrected visual acuity (BCVA), IOP and the number of antiglaucoma medications used. Postoperative data, recorded at each follow-up visit, are IOP, BCVA, the number of antiglaucoma medications, corneal graft status, the presence and nature of complications, and the necessity for reintervention. The IOP is measured by Goldmann applanation tonometry (Haag-Streit AG, Koeniz, Switzerland) or with the iCare rebound tonometer (Tiolat Oy, Helsinki, Finland) when applanation tonometry cannot be performed.

The surgical procedures were performed in the operating room, under retrobulbar anesthesia with a mixture of 3 ml of lidocaine 4% and 1 ml of bupivacaine 1%. We used 2% methylcellulose as a coupling agent to facilitate the slow and continuous movement of the cyclophotocoagulation of the probe and to enhance the transmission of laser energy to the concerned tissues. MP-TSCPC was performed using an MP P3 handpiece with the Iridex Cyclo G6 (IRIDEX Laser System). The parameters used were a power of 2000 mW and a duty cycle of 31.35% (an "on" period of 0.5 ms and an "off" period of 1.1 ms). The probe was applied on the sclera, with the notch at one mm from the limbus, for a period of 90s per hemiglobe, using moderate, but firm pressure, in a continuous sweeping motion and avoiding the 3 and 9 o'clock meridians, filtering blebs, areas of scleral thinning, and glaucoma drainage devices. All surgeries were performed by the same surgeon, MZ. Postoperative treatment included topical dexamethasone 0.1%, 4 times daily and cyclopentolate 1%, 2 times daily, as well as the preoperative topical antiglaucoma medication (but discontinuing oral acetazolamide). Therapy was adjusted at each follow-up visit. Patients were examined the next day after the intervention and then at 1, 3, 6, 9 and 12 months. After one year, follow-up visits were scheduled at a 3-months interval. For the inclusion of patients in the study, a follow-up of minimum of 12 months was required.

The primary outcome measure was the successful decrease of IOP. Therapeutic success at any time point was defined as an IOP greater than 5 mm Hg and lower than 21

mm Hg or a reduction in IOP of more than 30% compared to the baseline value. Hypotonia, defined as IOP lower than 5 mm Hg was considered a therapeutic failure. Secondary outcome measures were the number of glaucoma medications, the BCVA, the corneal graft status, and the occurrence of complications. All the data from the study will be analyzed using IBM SPSS Statistics 25 and illustrated using Microsoft Office Excel/Word 2013. Quantitative variables were tested for normal distribution using the Shapiro-Wilk Test and were written as averages with standard deviations or medians with interquartile ranges (IQR). Qualitative variables were written as counts or percentages.

Quantitative variables with non-parametric distribution were tested between intervals using Wilcoxon/Friedman tests. Qualitative variables were tested between intervals using Cochran's Q test. Post-hoc Dunn tests with Bonferroni correction were made to further detail the results obtained in the initial tests. A significance level of 0.05 was selected as a threshold for statistical significance.

The preliminary results suggest that MP-TSCPC is a treatment method with good efficacy in cases of glaucoma occurring in the setting of previous PK. The procedure has a good safety profile, minimally affecting visual acuity and the corneal graft.

The final data will be soon revised and the manuscript will be submitted for publication.

III.3. Final remarks

This habilitation thesis presents my professional and academic career and the most important scientific achievements during the postdoctoral thesis. Also, some of the main scientific projects to be finalized in the nearest future are revealed.

Undoubtedly, acquiring the habilitation certificate upon the validation of this thesis is of major importance to me. It will represent not only the recognition of my entire work, but it will allow me to progress even more in my professional, academic/teaching, and scientific career.

Last but not least, I would like to use this opportunity to express my gratitude, once again, to all my mentors and partners during the years.

SECTION IV
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