

EVER 2009



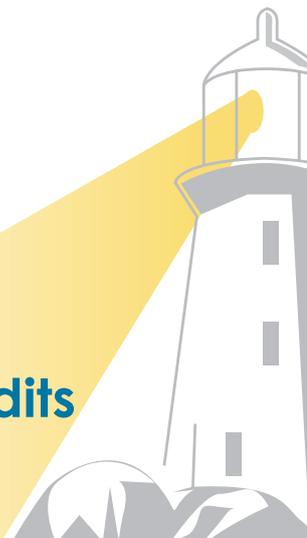
European Association for Vision and Eye Research

September 30 - October 3, 2009
Portoroz, Slovenia

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Concordia Visu

21 CME credits





EVER travel grants

The following 11 members have received a travel grant from the EVER Sections:

- **Anatomy/Cell Biology: PETROVSKI Goran, Hungary**
Clearance of dying cells in the retina - relevance to age-related macula degeneration (4332)
- **Cornea / Ocular Surface: DE LA PAZ Maria, Spain**
Ocular surface findings in patients with congenital aniridia (303)
- **Glaucoma: TODANI Amit, Saudi Arabia**
Measurement of IOP with radiowave telemetry (225)
- **Immunology/Microbiology: JAKOB Eva, Germany**
Three years experience of Quantiferon®Gold testing (406)
- **Lens and Cataract: KERNT Marcus, Germany**
Coenzyme Q10 prevents human lens epithelial cells from light-induced apoptotic cell-death by reducing oxidative stress and stabilizing BAX/Bcl-2 ratio (337)
- **Molecular Biology/Genetics/Epidemiology: VAN BERGEN Tine, Belgium**
Is inhibition of VEGF165 sufficient to inhibit scar formation after trabeculectomy? (2442)
- **Neuro-ophthalmology/Strabismology/Paediatric/History: ANGELI Raffaella, Italy**
Preterm and at term children: morphological and functional analysis of optic nerve and visual pathway with OCT, HRT and pVEP (2262)
- **Pathology/Oncology: DOPIERALA Justyna, UK**
Heterogeneity in uveal melanoma assessed by multiplex ligation-dependent probe amplification (MLPA) (3263)
- **Physiology/Biochemistry/Pharmacology: VIIRI Johanna, Finland**
Cis-urocanic acid suppresses UV-B-induced interleukin-6 secretion and cytotoxicity in human corneal and conjunctival epithelial cells in vitro (271)
- **Retina/Vitreous: MENDRINOS Efstratios, Switzerland**
Internal limiting membrane surrounding idiopathic stage 4 macular hole contains bundles of actin microfilaments (461)
- **Vision Sciences/Electrophysiol./Physiological Optics: STRASSER Torsten, Germany**
Development of a software for the exchange of electrophysiological data of vision with PACS systems based on DICOM (370)



EVER listed countries travel grants

The following 10 members from listed countries will receive a travel grant from the EVER Sections:

- **Russia - Andrey KACHANOV**
LASIK surgery for myopia in Chinese patients (334)
- **Serbia - Svetlana STANOJLOVIC**
Immune regulatory effect of rapamycin and cyclosporine A on experimental corneal allograft survival (329)
- **Hungary - Anita GARAS**
RTVue Fourier-domain OCT: reproducibility of RNFLT and macular thickness measurements (2253)
- **Czech Republic - Vladimir HOLAN**
Treatment of ocular surface injuries by the transfer of limbal and mesenchymal stem cells growing on nanofibrous scaffolds (205)
- **Slovenia - Spela STUNF**
Ultrastructure of anterior lens capsule in uveitic cataract (412)
- **Poland - Marzena GAJECKA**
Gene screening at the 13q32 keratoconus locus (2244)
- **India - Sumita SETHI**
A Western blot analysis of P-glycoprotein in retinoblastoma (3262)
- **Latvia - Liga RADECKA**
Wet AMD treatment with ranibizumab - structural and functional changes during treatment (420)
- **Slovenia - Natasa VIDOVIC**
The role of VEGF in intermediate uveitis patients (414)
- **Lithuania - Loresa KRIAUCIUNIENE**
Optico-anatomical changes of premature children eyes (358)



Alta Eficacia Tecnología SL, Spain, travel grant

The following paper in the VEP section will receive a travel grant for best paper from Alta Eficacia Tecnología

- **Franziska RAUSCHER - UK**
Differential effects of ageing on foveal and peripheral colour vision (3123)



TFOS awards 2009

The following three researchers have received a travel award from the Tear Film and Ocular Surface Society for the three best abstracts submitted to EVER 2009 by young researchers in the cornea section:

- **Jared AMENT:** Boston, USA
- **Stefano BARABINO:** Genoa, Italy
- **Antonia DEMESTICHA:** Heraklion, Greece

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▪ 1001

Corneal surface regeneration: epithelial sheets to epithelial crypts

Harinder DUA

Ophthalmology and visual sciences, University of Nottingham, Nottingham

The corneal surface is the most important 123 square millimetres of the body's surface. Its health is paramount for sight. Due to its exposed nature it is vulnerable to environmental insults causing minor to excessive epithelial cell loss. Large epithelial defects, with an intact limbus heal by the formation of 3 to 6 convex sheets that migrate centripetally, meet adjacent sheets and undergo contact inhibition to form geometric shapes. These finally close by the formation of 'Y' shaped contact lines (Rule 1). When the limbus is partially involved, there is a preferential healing of the limbus by circumferential migration of tongue shaped sheets from the remaining intact limbus. These meet to cover the limbus (Rule 2) and subsequent healing occurs as in rule 1. On occasions conjunctival epithelium migrates across the limbus resulting in partial limbal deficiency. When the entire limbus is affected, total conjunctivalisation of the cornea occurs (Rule 3). Within sheets, individual cells migrate in a predominantly clockwise whorl pattern (Rule 4). Physiological and wound healing related cell turnover is believed to occur from stem cells (SC) located at the limbus. Specialised anatomical structures, termed Limbal Epithelial Crypts (LEC) are associated with some limbal palisades and present all the hallmarks of a SC niche. SC characteristics are also seen in the peripheral cornea adjacent to a palisade with a LEC. Recent evidence suggests that the limbal SC play a critical role in wound healing but may have only a marginal role physiological corneal epithelial homeostasis.



▪ 1002

Hans Goldmann (1899 – 1991) – the man who coined the daily practice of ophthalmology worldwide during half a century until today

*Balder GLOOR
Zürich*

Goldmann presented 1937 a slit lamp, which allowed for the first time to examine the whole eye from the surface of the cornea to the fundus on the sitting patient with ease. Motion in three dimensions was possible with the fingertips of one hand. A reduction of the angle between observation and illumination beam allowed to use contact lenses with the free hand to investigate 360° of the chamber angle. Since then the difference between angle closure and open angle glaucoma is established. The illumination system of the slit-lamp was brought in vertical position in 1958, the Haag-Streit slit lamp 900 was born. It sets, with a few modifications, still the standard. In the meantime the Goldmann cupola perimeter was developed (1945), which allowed the standardization of background and target illumination for the first time. The applanation tonometer was introduced in 1954, again a standard instrument until today. Meanwhile these instruments determine the daily practice, for Goldmann they were, with other inventions, the tools to measure patho-physiologic processes and to understand diseases. Most determined was Goldmann in glaucoma research: Gonioscopy, perimetry to follow the increase of glaucomatous damage, detection of the aqueous veins, measurement of the production of the aqueous by fluorescein-dilution curves, determination of the outflow facility and pseudofacility, establishment of the “Goldmann-Formula”, tonometry by applanation and finally stereochronoscopy, these were the steps that unveiled glaucoma.

Goldmann, a most gifted fascinating personality, born in Komotau in Bohemia, grew up in Prague, was educated in arts, mathematics and physics, was trained by von Tschermak-Seysenegg and Elschning in Prague, then by Siegrist in Berne. He became Siegrist's successor in Berne 1935, this in spite of dangerous feuds with Alfred Vogt on the etiology of the glass blower cataract. He was granted Swiss citizenship 1936. He was an exceptionally great teacher for the students. He became Rector magnificus of the University of Berne in 1965. He retired in 1968, having chaired the department for 44 years. He remained active in research until his late eighties.

1991 ophthalmology lost one of his greatest genius.



▪ 1003

From intravitreal cell-enclosed drug therapy to low-(brain-)pressure-glaucoma

Jost JONAS

Department of Ophthalmology, Faculty of Clinical Medicine Mannheim of the University Heidelberg, Mannheim

Purpose To present potentially new developments in the intraocular drug delivery for therapy of retinal diseases as well as new development for the discussion of the pathogenesis of glaucomatous optic neuropathy.

Methods The advantages and limitations of the current intraocular drug therapy will partially be discussed. Additionally, morphologic findings of the optic nerve head assessment in glaucoma and of measurements of the lumbar cerebrospinal fluid pressure (CSF-P) will be presented.

Results Analysis of morphologic differences between glaucomatous versus non-glaucomatous optic nerve damage suggests that high-pressure glaucoma and normal-pressure glaucoma may share some aspects in their pathogenesis. Correspondingly, the CSF-P data suggest that normal-pressure glaucoma may be associated with a low brain pressure.

Experimental studies on the intraocular cell-based drug delivery suggest that these cells survive for several weeks in the vitreous cavity and continuously produce substances, e.g. glucagon-like peptide 1 and endostatin.

Conclusions The preliminary data suggest that intraocular cell-based therapy is potentially useful for the treatment of intraocular diseases. Additionally, the CSF-P measurements suggest that a low brain pressure may potentially be connected with glaucomatous optic neuropathy.



▪ 2001

Pathogen interactions in the pathogenesis of endophthalmitis

Michael GILMORE

Harvard Medical School, Schepens Eye Research Institute, Boston

Endophthalmitis is an often blinding complication of penetrating globe injury, and an unfortunate consequence of common ocular surgeries, which requires immediate intervention. Although penetrating injuries are more likely to be associated with infectious complications, because of the number of ocular surgeries performed, postoperative endophthalmitis is its most common form. Given the challenge of maintaining a sterile field in ocular surgery, the fact that many surgeries are sutureless leaving a potential portal of entry, and the reported rates of bacterial contamination of irrigation fluid, it is perhaps surprising that the rate of infection in most centers is between 1 and 3 per 1000 surgeries.

Although postoperative endophthalmitis rates are low, they appear to be increasing in the US and potentially elsewhere. This may relate to increased age of patient at the time of surgical intervention, increased rates of underlying illness, especially adult-onset diabetes, and other factors. The development of new knowledge in several key areas is setting the stage for new and better strategies for infection treatment and prevention. It is now appreciated that the immune privilege of the posterior segment creates a highly permissive environment for the growth of microbes of even low virulence, such as coagulase negative staphylococci – a leading cause of postoperative endophthalmitis. The widespread use of antibiotic has selected for resistant strains of these and other organisms with increased virulence, including the ability to form tenacious and antibiotic refractory biofilms. We are beginning to understand the molecular basis for immune privilege in the posterior segment as it relates to the ability of innate immunity to contend with infecting microbes. We are also beginning to understand basic principles of the physiology of bacterial biofilms. The posterior capsule represents an important anatomical barrier to endophthalmitis, but many cases occur without known intraoperative breach. Studies now are attempting to identify bacterial properties, such as the production of tissue-destructive proteases, that promote translocation of the microbes through an intact capsule and into the posterior segment. The contribution of innate immune privilege of the posterior segment, microbial virulence, and biofilm formation to endophthalmitis will be presented.



■ 3001

Age-related macular degeneration; an Alzheimer's disease in the eye?

Kyoko OHNO-MATSUI

Tokyo Medical and Dental University, Ophthalmology and Visual Science, Tokyo

Purpose One of the earliest signs of age-related macular degeneration (AMD) is the formation of drusen beneath the retinal pigmented epithelium (RPE). Recent proteome analysis demonstrated that amyloid β ($A\beta$), which is a major component of senile plaques in the brains of patients with Alzheimer's disease. The purpose of our study was to clarify the role of $A\beta$ on the pathogenesis of AMD.

Methods The effect of $A\beta$ on the expression of angiogenesis-related factors, cytokines, and the modulators of complement pathway in cultured human RPE cells was investigated. Ocular findings in neprilysin gene-disrupted mice, which lead to an increased deposition of $A\beta$, were evaluated.

Results $A\beta$ treatment induced a marked increase in VEGF as well as a marked decrease in pigment epithelium-derived factor (PEDF) in RPE cells. Electron microscopy of the senescent neprilysin gene-disrupted mice revealed the degenerated RPE cells with vacuoles and basal deposits formation. $A\beta$ did not directly affect the expression of modulators of complement pathway in RPE cells, but increased the production of MCP-1. Co-cultures of RPE cells and macrophage/microglia in the presence of $A\beta$ significantly increased the expression of the activator of complement alternative pathway; factor B by RPE cells through IL-1 β /TNF- α secreted by macrophages/microglia. $A\beta$ binds to the inhibitor of alternative pathway; factor I and inhibited its ability to cleave C3b to inactivated iC3b.

Conclusions $A\beta$ has a critical role in the pathogenesis AMD. Combined mechanism exists for $A\beta$ -induced activation of the complement alternative pathway in subretinal space; cytokine-induced up-regulation of activator factor B and dysfunction of the inhibitor factor I. Un-regulated complement activation leads to a low-grade, chronic inflammation in subretinal tissue, which could cause AMD development.



▪ 3251

Women in Ophthalmology: From bench to boardroom

Adrienne GRAVES

President and CEO, Santen Incorporated

Dr. Adrienne Graves began her career as a visual scientist, with stops at Brown University, Harvard Medical School, University of Michigan, and University of Paris. Her passion for studying the visual system and the pathophysiology of disease processes led her to the pharmaceutical industry. After positions in R&D at Alcon, she is now President/CEO of Santen's US operations. Dr. Graves credits her time in Paris for expanding her horizons in many ways, including an appreciation for cultural diversity. This is especially important in her role as a Corporate Officer for Santen, a global ophthalmic pharmaceutical company based in Japan.



▪ 3002

Role of the vascular endothelial cell in uveitis

Justine SMITH

Casey Eye Institute, Oregon Health & Science University, Portland

Of all the forms of inflammatory eye disease, posterior uveitis is the most likely to result in irreversible loss of vision. This disease results from: autoimmune disease; infection, most commonly toxoplasmosis; and in rare cases, B cell lymphoma. Patients with posterior uveitis frequently demonstrate clinical involvement of the retinal vasculature. Studies of rodent experimental autoimmune uveoretinitis conducted by other groups show that the lymphocytes that control this disease model access the retina via the retinal blood vessels. We have developed methods to isolate vascular endothelial cells from the retina of human cadaver donors; lymphocytes migrate readily across monolayers of these cells. In separate studies, we have observed that in comparison to other endothelial cell subtypes, retinal endothelial cells are relatively susceptible to infection with *Toxoplasma gondii* tachyzoites; increased binding of tachyzoites to the retinal endothelium may contribute to this susceptibility. We have also detected the expression of B cell lymphoid chemokines by vascular endothelial cells, as well as malignant lymphocytes, in primary central nervous system B cell lymphoma. Profiling analyses of the human retinal vascular endothelial cell, using gene expression microarray and shotgun proteomics, may help to clarify the molecular mechanisms that mediate interactions between the retinal vascular endothelium and lymphocytes or microbes in posterior uveitis. As the “gate” of the retina in posterior uveitis, the retinal endothelial cell holds much promise as a target for highly specific therapies of this disease.



▪ 4001

Unravelling the mysteries of ocular lymphomas

Sarah COUPLAND

Pathology, University of Liverpool, Liverpool

Purpose There has been a bewildering evolution in the nomenclature of lymphomas, including ocular lymphomas. Confusing as this may seem, this is a reflection of impressive advances in our understanding of lymphoma pathogenesis, mainly as a result of immunohistology and molecular biological research. This overview will summarise the main successes of ocular lymphoma research and its translation into clinical practice.

Methods The WHO lymphoma classification is based on the morphology, immunophenotype and genotype of tumour cells and their relationship to a putative cell of origin. Most ocular adnexal lymphomas (OAL) are low-grade B-cell lymphomas (EMZL), thought to arise from marginal zones of lymphoid follicles. Their development is associated with chromosomal abnormalities, autoimmune disease & microorganisms. Traditionally, OAL have clinically been staged using the Ann Arbor system, which has many shortcomings. The novel TNM-based staging system defines disease extent more precisely and allows for analysis of site-specific factors.

Results Retinal lymphoma has been vaguely subsumed under the term “primary intraocular lymphoma”: it is a high-grade B-cell malignancy, associated with a poor prognosis as a result of CNS disease. Immunophenotyping and genetic analysis suggest that retinal lymphomas arise from early post-germinal centre cells. Other less common intraocular lymphomas include primary choroidal lymphomas (a low-grade EMZL), primary iridal and ciliary body lymphomas, and secondary uveal lymphomas.

Conclusions Ocular lymphomas are heterogenous tumours with differing subtypes and varying clinical courses. Precise nomenclature together with exact subtyping and clinical staging is essential, to understand ocular lymphoma histiogenesis and to facilitate studies improving prognostication and therapy.

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■ 1211

Prevention of corneal graft rejection

*PLEYER U
Berlin*

ABSTRACT NOT PROVIDED

■ 1212

Principles of immunosuppression in uveitis

*WILLERMAIN F, CASPERS L
Ophthalmology, CHU St-Pierre, Bruxelles*

Non infectious uveitis is a heterogeneous group of diseases mediated through autoimmune and autoinflammatory mechanisms. It is thus crucial to perform a complete work-up to characterise the disease and eventually find a precise aetiology or a systemic associated condition. When the inflammation is bilateral and the vision threatened, systemic drugs are usually proposed. Despite tremendous progress in the understanding of the disease, treatments are generally based on the administration of non specific immunosuppressive molecules. Currently, high doses oral corticosteroids are first given, followed by a slow tapering of the dosage. If this strategy does not lead to disease control, a steroid-sparing agent should be considered. Antimetabolites, T-cell inhibitor and alkylating agents will be chosen (alone or in combination), depending on the severity of the disease and patients general status. Recently the development of biologic agents offers the possibility to target various specific molecules important in non infectious uveitis development. Nowadays, anti-TNF α have been mostly tested with encouraging results. However, it is likely that different uveitis subtypes would require different biologic agents. In the future, the growing production of specific inhibitors might lead to a more tailored approach of uveitis treatment.

■ 1213

The present role of corticosteroids in uveitis

*KHAIRALLAH M
Department of Ophthalmology, Fattouma Bourguiba University Hospital, Monastir*

Results Corticosteroids are the most widely used anti-inflammatory and immunosuppressant drugs in ophthalmology in general, and remain the mainstay of therapy for patients with uveitis. An infectious etiology for intraocular inflammation should be adequately excluded or appropriately covered with anti-infectious therapy before administration of corticosteroid therapy. Topical corticosteroids alone are usually effective in the management of anterior uveitis and have little activity against intermediate or posterior uveitis. Ocular adverse effects of topical steroid therapy mainly include ocular hypertension and cataract. The use of periocular steroid injections (subconjunctival, anterior or posterior subtenon, orbital floor) are important modalities in the management of anterior uveitis refractory to topical treatment and intermediate or posterior uveitis, particularly unilateral cases. Systemic corticosteroids remain the initial drug of choice for most patients with severe bilateral intermediate or posterior uveitis. Therapy is initiated with 1.0 to 2.0 mg/Kg of oral prednisone or prednisolone as a single morning dose, followed by a slow taper. Use of intravenous pulse steroid therapy is an important option in acute, severe, bilateral posterior segment inflammation. In several cases, the level of systemic steroid required to control the inflammation is too high and unacceptable. Immunosuppressive drugs as steroid-sparing agents are indicated in such cases. Intravitreal injection of triamcinolone acetonide and slow-release intraocular devices are therapeutic options that can be used in selected uveitis cases refractory to conventional therapy and biologic agents.

■ 1214

Traditional immunosuppressants for uveitis

DE SMET M

ABSTRACT NOT PROVIDED

■ 1215

Interferon-alpha for uveitis

BODAGHI B (1), DICK AD (2)

(1) Paris

(2) Bristol

ABSTRACT NOT PROVIDED

■ 1216

Immunosuppression in children (JIA)

BODAGHI B

Paris

ABSTRACT NOT PROVIDED

■ 1217

Infliximab (Remicade®) in uveitis: a review

ABU EL ASRARA (1), NERI P (2), HERBERT CP (3)

(1) King Abdulaziz University Hospital, Ophthalmology, Riyadh

(2) Polytechnic University of Marche, Eye Department, Torrette-Ancona

(3) University of Lausanne & Centre for Ophthalmic Specialised Care, Ophthalmology, Lausanne

Tumor necrosis factor (TNF)- α has been implicated as an important mediator in autoimmune ocular inflammatory disease pathogenesis as shown by animal studies and its detection in the ocular fluids of patients with uveitis. Blockade of TNF- α has emerged as one of the most promising therapies in autoimmune diseases including uveitis. Currently, there are three TNF- α antagonists: two monoclonal antibodies (infliximab and adalimumab) and a soluble receptor that binds soluble TNF- α (etanercept). Infliximab is a chimeric monoclonal antibody directed against TNF- α . It binds with high affinity to both the soluble and the membrane-bound TNF- α and inhibits a broad range of biologic activities of TNF- α . Binding to membrane TNF- α can mediate programmed cell death. Several studies reported that infliximab therapy was rapidly effective and safe treatment for refractory noninfectious uveitis including childhood uveitis and is indicated as rescue therapy for relapses of ocular inflammation or as maintenance therapy when conventional immunosuppression fails. It also allowed a reduction of corticosteroids and immunosuppressive drugs required to control the disease. However, repeated infusions are required to maintain long-term remission. Moreover, infliximab administration is costly and requires hospital admission. Adalimumab, fully humanized monoclonal anti-TNF- α antibody, was also found to be effective and safe therapy for the management of refractory noninfectious uveitis. Several studies reported that infliximab was more effective than etanercept in the treatment of refractory uveitis. Perhaps infliximab's ability to target membrane-bound TNF- α in addition to the soluble form may contribute to its increased efficacy in comparison with etanercept for uveitis.

■ 1218

Final results of European Remicade® RESCU study

DE SMET M

Lausanne

ABSTRACT NOT PROVIDED

■ 1219

Adalimumab (Humira®) therapy for uveitis

NERIP

Azienda Ospedaliero-Universitaria "Umberto I-GM Lancisi-G Salesi", Ancona

Purpose To review the current Literature and to describe the experience of a tertiary referral centre on Adalimumab (Humira®) therapy for uveitis.

Methods The current literature is reviewed and the experience of a tertiary referral centre is reported.

Results Chronic non-infectious uveitis (NU) remains one of the most challenging problems in ophthalmology. Often, early and aggressive treatment is needed for a good visual acuity outcome. Local and oral corticosteroids remain the first line of treatment, even though side effects, such as glaucoma, cataract and Cushing Syndrome, can be serious and not tolerable. Therefore, a variety of immunosuppressive agents are in use, such as Cyclophosphamide, Methotrexate, Cyclosporin A, Azathioprine and Mycophenolate mofetil. Recently, new types of drugs, called "biologic agents", are available. In the model of experimental autoimmune uveitis (EAU) it has been demonstrated that tumornecrosis factor-alpha (TNF- α) may play a key role in uveitis. Beside other anti- TNF- α drugs, Adalimumab (Humira®) is the newest biologic drug available. Adalimumab is a recombinant human IgG1 monoclonal antibody to TNF- α that blocks the TNF- α biologic activity. Adalimumab seems to control uveitis unresponsive to the traditional immunosuppressive agents and, moreover, its subcutaneous administration makes the procedure easier.

Conclusion The data in the literature suggest that Adalimumab can be an effective and safe therapy for the uveitis, by controlling inflammation of the eye. The duration of treatment have still to be investigated in larger studies and further trials are mandatory to validate the preliminary data.

■ 121b

Optimised monitoring of inflammation suppressive therapy (IST) in uveitis

HERBERT CP (1, 2)

(1) *Inflammatory and Retinal Eye Diseases, Centre for Ophthalmic Specialised Care (COS), Lausanne*

(2) *University of Lausanne, Lausanne*

Purpose The array of inflammation suppressive therapies (IST) has increased tremendously in the last two decades including the availability of biological molecules with potent immunomodulatory activities as well as new immunosuppressive agents. In parallel measuring methods for intraocular inflammation have become available allowing much more accurate monitoring of the evolution of inflammation and its response to therapy. In addition to the traditionally used fluorescein angiography (FA), laser flare photometry (LFP), indocyanine green angiography (ICGA) and optical coherence tomography (OCT) are among the new investigational methods that have become available, each of them allowing us to establish inflammatory activity with increased precision and to explore compartments previously poorly accessible.

Methods The advantages of each method will be put forward. Illustrative cases will be taken as examples to show the degree of precision obtained by combining the different methods presently at our disposal for the follow-up and monitoring of inflammation suppressive treatment.

Results These cases will show that the presently available tools for optimal monitoring of intraocular inflammation allow the clinician to be better aware of the level of inflammatory activity and to better adapt his treatment.

Conclusion Not only the availability of potent new therapies but also the possibility to follow more precisely intraocular inflammation with new precise devices has certainly changed the outcome of uveitis cases in recent years.

■ 121a

Mycophenolate mofetil in uveitis

NERIP

Azienda Ospedaliero-Universitaria "Umberto I-GM Lancisi-G Salesi", Ancona

Purpose To review the current Literature and to describe the experience of a tertiary referral centre on the use of mycophenolate mofetil (MMF) treatment in uveitis.

Methods The current literature is reviewed and the experience of a tertiary referral centre is reported.

Results The long-lasting remission in several systemic diseases, such as Crohn's disease, severe atopic dermatitis, Wegener's granulomatosis and microscopic polyangiitis, rheumatoid arthritis, pemphigus vulgaris, and psoriasis, have been proven. Recent publications have recently confirmed the satisfactory control of uveitis with MMF in a large cohort of patients. Moreover, the long-term control of cystoid macular oedema (CMO) unresponsive to the traditional therapy has been described, as well as for the choroidal neovascularization (CNV).

Conclusion Non-infectious uveitis is one of the leading causes of visual impairment in ophthalmology. Steroids can control such disease, even though a long-term treatment is not recommended: several complications, such as high blood sugar level, osteoporosis, blood cell abnormalities, cataract and glaucoma, can occur. MMF is a reversible, non competitive, selective inhibitor of the de-novo pathway of purine synthesis; mycophenolic acid has a strong effect to Type II isoform of inosine monophosphate dehydrogenase enzyme, providing a stronger cytostatic effect on lymphocytes than on other cells types, with minor action to Type I expressed in most other cells. The specific action of MMF on selected targets makes it a promising drug for the control of non-infectious intraocular inflammations.

■ 121c

The future in immunomodulation

DE SMET M

Lausanne

ABSTRACT NOT PROVIDED

■ 1231

Pitfalls in statistics and how to avoid them - part 1

KIVELÄ T

Helsinki University Central Hospital, Helsinki

Purpose To highlight some pearls and pitfalls in reporting summary statistics and in using common statistical tests.

Methods Personal experience of the author as a writer, reviewer and editorial board member especially for the EVER Journal, Acta Ophthalmologica, is used to highlight some of the most common possibilities for improvement in using basic statistics.

Results The items discussed, based on examples from the literature, include:

1. how to avoid giving a false impression of precision when reporting key results and how to communicate effect size;
2. how to differentiate statistical from clinical significance and how to communicate statistically nonsignificant results when writing the manuscript;
3. recognising the common problem of making multiple comparisons and adjusting for them; and
4. differentiating ordered and unordered categorical data and choosing the proper statistical test accordingly.

Conclusion Clear reporting and analysis of basic statistics is not difficult as long as one is aware of a few basic rules, which will be discussed in this talk. Putting them into action may improve your manuscripts.

■ 1232

Tips and tricks for writing a manuscript

DAMATO B

Liverpool

Purpose The aims of this presentation are to highlight some pearls and pitfalls in writing scientific articles.

Methods It is not possible to write a good manuscript unless you have a good message to share. If you are reporting research results this means that you will need to have conducted the research properly, with good statistics, measures to avoid bias, complete data and, of course, an important hypothesis to test. If possible, expert advice should be obtained before the study is started so as to ensure that the trial design is appropriate and that the statistics are correct.

Results The key to writing a good manuscript is to divide the paper into sections and to sub-divide each section into components, making sure that the sequence of ideas is in the correct order. The internet is full of good advice on how to write a good manuscript. This information be summarized in this presentation, which will also guide participants to useful sources of information.

Conclusion Writing a manuscript is not difficult and should be a pleasurable and rewarding experience as long as one follows a few simple rules, which will be discussed in this talk.

■ 1233

Pitfalls in statistics and how to avoid them - part 2

KIVELÄ T

Helsinki University Central Hospital, Helsinki

Purpose To highlight some pearls and pitfalls in reporting statistics related to the eye.

Methods Personal experience of the author as a writer, reviewer and editorial board member especially for the EVER Journal, Acta Ophthalmologica, is used to highlight common issues specific for reporting eye-related statistics.

Results The items discussed, based on examples from the literature, include:

1. proper ways of summarising and comparing visual acuities; and
2. proper ways of analysing data collected from both eyes of the patients.

The need to find a statistician well versed in ophthalmic research at the planning stage of a study is stressed

Conclusion Correct reporting and analysis of data related to the eye is not difficult as long as one is aware of a few basic rules, which will be discussed in this talk. Putting them into action helps to ensure that the conclusions the readers draw from your study are proper.

■ 1251

Diabetic retinopathy

POURNARAS CJ, MENDRINOS E

Department of Ophthalmology, Faculty of Medicine, University Hospitals of Geneva, Geneva

Purpose To describe the clinical stages of diabetic retinopathy (DR) and diabetic macular edema (DME), their complications and treatment.

Methods Diabetic retinopathy is currently classified into several groups: minimal non-proliferative, moderate non-proliferative, severe non-proliferative and proliferative with or without high-risk characteristics. To characterize the severity of DME and for treatment guidelines, the term clinically significant macular edema (CSME) is used. CSME is further classified into focal or diffuse.

Results The role of panretinal photocoagulation in reducing the risk of severe visual loss related to proliferative diabetic retinopathy has been demonstrated by prospective large scale randomized studies (DRS, ERTDS). Similarly, the ETRDS study showed the benefit of focal/grid laser photocoagulation in reducing the risk of moderate visual loss in CSME. In the last years, promising results have been published on the use of intravitreal (IVT) injections of triamcinolone acetonide, anti-VEGF agents, and on the use injectable sustained release steroid implants in terms of visual gain and reduction of central macular thickness. Laser photocoagulation was demonstrated more effective than IVT triamcinolone alone for the treatment of diabetic macular oedema. The risks and benefits of novel treatment modalities and their efficacy and safety should be further evaluated in prospective well designed studies. Vitrectomy is indicated in selected cases.

Conclusion Diabetic retinopathy is characterized by gradually progressive alterations in the retinal microvasculature leading to neovascularization, vitreous hemorrhage, retinal detachment and macular edema. Several treatment modalities are still investigated. Laser photocoagulation is the standard of care.

■ 1253

Age related macular degeneration

VOIGT M

Paris

ABSTRACT NOT PROVIDED

■ 1252

Pigmented lesions

DESJARDINS L

Paris

Purpose The aim of the course is to describe symptoms, clinical findings and main diagnostic procedures for pigmented fundus lesions

Methods Asymptomatic lesions include choroidal naevi, melanocytomas and congenital hypertrophy of the pigment epithelium. Symptomatic lesions include combined hamartomas of the retina and pigment epithelium, suspicious choroidal naevi, choroidal melanomas and subretinal hematomas

Results For each of the lesions fundus aspects will be described as well as the results of ultrasonography, fluorescein angiograms, OCT and MRI. With the symptoms, clinical findings and results of ancillary tests diagnosis and differential diagnosis will be discussed

■ 1254

Retinal dystrophies

HAWLINA M

University Eye Hospital, Ljubljana

Purpose Review of typical clinical pictures and gene defects associated with most prevalent retinal dystrophies such as retinitis pigmentosa, Stargardt's macular dystrophy, Best's macular dystrophy, cone dystrophy, congenital stationary night blindness, pattern dystrophy and some rarer types of dystrophies.

Methods Clinical methodology to diagnose retinal dystrophies is based on conventional perimetry, microperimetry, autofluorescence imaging, OCT and electrophysiology. The use of these methods will be outlined in characteristic cases. Genetic testing is necessary to characterise the genetic cause of these diseases as clinical heterogeneity is large.

Results Autofluorescence imaging and OCT non-invasively reveal typical morphological patterns of different retinal dystrophies, for which fluorescein angiography is not necessary. Different electrophysiological methods (photopic/scotopic ERG, pattern ERG, multifocal ERG, visual evoked potentials) determine function of retinal layers and the macula. Residual retinal function and progression of the disease can thus be objectively assessed and followed-up. In initial stages when retinal changes may not be visible, electrophysiological features can reveal the site of dysfunction and dissociate it from putative optic nerve disease.

Conclusion The residents should be aware of the differential diagnosis of retinal dystrophies and be able to dissociate between them. Also, typical genetic transmission and genetic counseling is important for the information that is given to the patient. Residents should be aware of treatable associated features of retinal dystrophies such as cataract or cystoid macular edema or glaucoma.

■ 1255

What do I need to improve my glaucoma skills?

BRON AM

Ophthalmology, University Hospital, Dijon

Purpose Many new devices and technologies are available nowadays in the diagnosis and the treatment of glaucomas. However for young practitioners, it is mandatory to concentrate on symptoms and clinical signs.

Methods In this presentation I will assess the place of the clinical examination in the overall management of glaucoma. In clinical practice many clues for glaucoma are found with simple instruments; a slit-lamp, a gonio-lens and a fundus lens. I will provide a frame, a sort of check list in order to build a diagnosis with the signs found during the clinical examination.

Conclusion A moderate knowledge about ocular anatomy and physiology will lead the young resident through an easier diagnosis of glaucoma. Looking for the relevant symptoms and signs during the clinical examination will lead the young practitioner to a good accuracy in the diagnosis of glaucoma.

■ 1256

Refraction and refractive surgery

COCHENER B

Brest

ABSTRACT NOT PROVIDED

■ 1321

Corneal bacterial infections

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(2) Department of Ophthalmology - University Hospital, Strasbourg

Purpose To present the main causes of corneal bacterial infections and suggest an appropriate management.

Methods Bacterial keratitis is the most common cause of infected corneal ulceration. The epidemiology of corneal bacterial diseases and their risk factors will be studied. The initial presentation is important to determine the potential stain responsible for the infection and to assess the overall severity of the disease. Local and general criteria evaluations are needed to adapt the treatment.

Results Specific signs to identify bacteria remain rare but some signs can help to determine the first line treatment. However laboratory methods remain the only way to diagnose a corneal bacterial disease with certainty. However the aspect of the infiltrate, the intraocular involvement and the condition of the patient may influence the outcome. The treatment is based on an initial broad-spectrum antibacterial therapy with secondary adaptation to the identified bacteria.

Conclusion Multiple microorganisms are involved in corneal infections. Local and systemic risk factors have to be identified to treat these diseases in emergency still considered as a leading cause of visual loss.

■ 1323

Epidemiology of viral infections

LABETOUILLE M

Hôpital de Bicêtre, Ophthalmologie, Kremlin Bicêtre

The three main causes of viral keratitis are Herpes simplex virus (HSV), varicella-zoster virus (VZV) and adenovirus (ADV). Corneal HSV infection is a frequent cause of severely impaired visual acuity. Despite the effectiveness of currently available antiviral drugs, the incidence of herpetic corneal events does not tend to decrease in the general population, as recently showed in a nationwide epidemiological study performed in France. On the other hand, corneal transplantations relative to herpes keratitis tend to become rarer thanks to the effectiveness of preventive oral treatment. Management of herpes keratitis could thus be improved by a better knowledge of epidemiological data. For example, children and atopic patients are now identified as groups of patients with increased risk of severe herpetic disease. The epidemiology of VZV-related keratitis may drastically vary in the future, due to the generalization of the vaccination against chickenpox/herpes zoster, with a probable increase in the next decades, followed by a progressive and durable decrease. Finally, outbreaks of ADV-related keratitis are now less frequent than fifty years ago, thanks to the more rigorous hygiene in the general population.

■ 1322

Infectious crystalline keratopathy

DUA HS

Division of Ophthalmology and Visual Sciences, Nottingham

Purpose To provide the definition, clinical features, diagnosis and management of infectious crystalline keratopathy (ICK).

Methods Personal experience, experimental study on ex-vivo ICK and literature review

Results ICK can be defined as microbial infection of the cornea in the absence of the host immune response. It can be caused by a variety of microbes including bacteria and fungi. It is characterised by corneal infiltrates in the form of branching or arborescent patterns of needles, snowflakes or fern shapes. This pattern is determined by the compactness of the corneal lamellae. Clinically there is minimal host inflammatory response although there can be areas of suppuration remote from the ICK. The epithelium is usually intact, symptoms are mild and it runs a chronic indolent course. Corneal hypoesthesia and corticosteroid use are risk factors. Diagnosis is based upon clinical examination in conjunction with careful history taking. The presence of branching crystalline deposits located in the stroma often near suture sites and following corneal surgery should raise suspicion. Intensive topical antibiotics, keratectomy of the affected site to excise lesion if possible, cessation of steroid medication or repeat corneal graft are aspects of management of ICK.

Conclusion ICK is a rare but difficult to treat condition. Diagnosis is based on history and clinical features. Sample (scrape) or biopsy can be taken for culture and sensitivity of organisms involved. Treatment is difficult and surgery may be required to eliminate the infected locus.

Commercial interest

■ 1324

Herpes and zoster keratitis

BOURCIER T

Strasbourg

ABSTRACT NOT PROVIDED

■ 1325

Fungal infections

*KESTELYN P
Gent*

ABSTRACT NOT PROVIDED

■ 1326

Parasitic corneal infections

*GICQUEL JJ
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The exposure of the eye directly to the environment renders it vulnerable to a number of uncommon infectious diseases caused by parasites. Once anatomical barriers are breached, host defences are often insufficient to prevent the infection from spreading locally. A fast identification and treatment of the involved microorganisms are necessary. Contact lens wear is associated with keratitis caused Acanthamoebae. Parasitic infections may also arise following bloodborne carriage of the microorganism to the eye or adjacent structures.

■ 1341

What is the consequence of retinal detachment on anatomy and function?

CRELIZOT C

Department of Ophthalmology - University Hospital, Dijon

Purpose To present the structural and functional consequences on retina after retinal detachment

Methods Author will show the consequences of experimental retinal detachment in animal models. These changes prevent retinal cells from a normal post-operative functioning. However, these conditions have to be differentiated from post-operative visual loss due to macular edema, long-standing subretinal fluid, epiretinal membrane or macular hole formation.

Results Retinal detachment leads to severe changes on retinal cells: outer segment shortening, fibrosis, glial proliferation. This situation is the target of neuroprotective treatment. By contrast, some situations where the bad recovery is due to an associated disease (edema, membrane...) illustrated by clinical cases can need surgical treatment.

Conclusion Bad functional recovery after retinal detachment can be explained by anatomic consequences on photoreceptors with subclinical fibrosis or some associated complications.

■ 1343

Primary vitrectomy in retinal detachment: is scleral buckling still indicated?

POURNARAS CJ

Department of Ophthalmology, Faculty of Medicine, University Hospitals of Geneva, Geneva

Purpose The surgical management of rhegmatogenous retinal detachment has evolved due to introduction of primary pars plana vitrectomy (PPV). The choice of primary vitrectomy has grown over recent years even for management of simple retinal detachment.

Methods Review of reports on primary vitrectomy treatment for rhegmatogenous uncomplicated retinal detachment. Evaluation of optimal indications of vitrectomy for specific types of retinal detachments.

Results Comparison of primary vitrectomy with scleral buckling had failed to demonstrate advantage of this method regarding anatomical and functional results. Vitrectomy avoids some of the complications associated with scleral buckling, such as diplopia, choroidal detachment, perforation of the sclera, abnormalities in the eyelid, but it carries higher risks of several other complications, including cataract formation in phakic eyes, glaucoma and other problems with tamponade, and new retinal breaks. Data from case series and randomized studies suggest that primary detachments in phakic eyes may be treated successfully with scleral buckling or vitrectomy, whereas vitrectomy appears to be preferable for corresponding detachments in pseudophakic eyes.

Conclusion The choice of primary PPV in new uncomplicated retinal detachment remains to the surgeon's discretion and skills due to lack of controlled randomized trials covering the large spectrum of the retinal detachment pathology.

■ 1342

Retinal detachment in phakic patients

CHIQUET C

Department of Ophthalmology, Grenoble

Purpose this review aims to summarize risk factors, preoperative evaluation and principles of operative methods of retinal detachment of phakic eyes

Methods Preoperative evaluation includes detailed examination of the retina, the identification of retinal breaks and classification of proliferative retinopathy. Main operative methods will be presented with ab externo or ab interno techniques.

Results after a detailed characterization of the retinal detachment, the more appropriate surgical methods will be explained for the search, the treatment (laser or cryotherapy), closure (scleral buckling materials) of retinal breaks, management of subretinal fluid (drainage) and the choice of the intraocular tamponade (gas or silicone).

Conclusion this review will discuss the main advantages of each surgical technique and examples of management will be presented (simple phakic detachment, associated cataract, giant tears, high myopia).

■ 1344

Retinal detachment after ocular trauma

JONAS JB

Department of Ophthalmology, Faculty of Clinical Medicine Mannheim of the University Heidelberg, Germany, Mannheim

Purpose To discuss the clinical characteristics and treatment options for retinal detachment after ocular trauma

Methods The various types of ocular trauma and the different types of retinal detachments occurring as a complication of ocular trauma will be presented and compared with each other

Results The surgical treatment options for retinal detachment after ocular trauma will be presented.

Conclusion Due to the high variability of ocular traumata and the resulting high variability in the type and extent of retinal detachments after ocular traumata, the decision on the type of surgical treatment will usually be rather individual according to the special clinical situation.

■ 1345

Retinal detachment after uveitis

DESMET M

ABSTRACT NOT PROVIDED

■ 1346

Glaucoma and retina

BRONAM

Ophthalmology, University Hospital, Dijon

Purpose Retinal and vitreoretinal surgeries frequently lead to an elevation of intraocular pressure (IOP) and/or glaucoma. Clinical presentations correspond to secondary glaucomas with an open-angle or with angle-closure.

Results Since myopia is a risk factor for retinal detachment and glaucoma as well, it is not surprising that these two disorders are often combined. Corticosteroids are widely used in retinal surgeries and the elevation of IOP related to the use of corticosteroids has long been recognized, whatever the route, topical, intravenous or intravitreal. Specific conditions such as Schwartz syndrome or Ghost-cell glaucoma are not very frequent. However scleral buckling procedures, injection of air, expansive gas and silicone oil may lead to severe elevations of IOP.

Conclusion The management of these secondary glaucomas can be difficult because of the inflammatory status of the eye and the recurrence of the retinal diseases. Clinical examination and particularly gonioscopy is the key issue to lead the clinician to the appropriate treatment.

■ 1361

Diagnosis of ophthalmic tumours

KIVELÄ T

Helsinki University Central Hospital, Helsinki

Purpose To summarise clinical methods used to diagnose ophthalmic tumours.

Methods Personal experience of the author as a member of the European Ophthalmic Oncology Group.

Results Conjunctival tumours are excised based on provisional clinical diagnosis or, if they are extensive, atypical or part of systemic disease such as lymphoma, first biopsied to obtain a histopathologic diagnosis. Useful methods to diagnose and stage conjunctival tumours are high frequency ultrasonography (US) or ultrasound biomicroscopy (UBM) to measure their thickness, in vivo confocal microscopy or impression cytology to chart their extent, and exfoliative cytology to get a provisional diagnosis. Ciliary body tumours are visualised by radical biomicroscopy, transillumination and indirect ophthalmoscopy with scleral indentation, supplemented with high frequency US or UBM. Binocular indirect ophthalmoscopy and US form the basis or diagnosing choroidal tumours. In addition to fluorescein and indocyanine green angiography in atypical cases, optical coherence tomography to detect subretinal fluid and autofluorescence to detect orange pigment are useful adjuncts in telling a small melanoma from a naevus. The mnemonic "To Find Small Ocular Melanomas" (from Thickness >2mm, subretinal Fluid, Symptoms, Orange pigment, Margin touching disc) is also useful in this respect. Clinical diagnosis of medium-sized to large melanomas is 99% accurate, whereas a fine needle or vitrectomy biopsy may be necessary to diagnose atypical tumours and is also used for cytogenetic analysis of uveal melanomas.

Conclusion Conjunctival tumours are mostly diagnosed histopathologically, whereas diagnosis of uveal tumours is usually based on clinical examination. While clinical diagnosis is usually reliable, biopsy of uveal tumours is increasingly used for prognostic purposes.

■ 1363

Management of retinal tumours

DESJARDINS L

Ophthalmology, Paris

Purpose To describe the clinical presentation, main diagnostic tools, differential diagnosis and management of retinoblastoma.

Methods Diagnostic modalities include fundus exam under general anesthesia with fundus pictures, ultrasonography and MRI. The more frequent differential diagnosis are coats disease and PHPV. Diffuse infiltrating retinoblastoma can be very difficult to identify. An overview of the different therapeutic modalities of retinoblastoma with their specific indications and guidelines for follow up of patients and genetic counseling will be given.

Results Unilateral massive retinoblastoma with no vision left is usually treated by enucleation while most bilateral retinoblastoma and 20% of unilateral can be treated by various conservative techniques including chemotherapy and chemothermotherapy, cryotherapy, plaque brachytherapy and external beam radiotherapy.

Conclusion Early diagnosis of retinoblastoma is still the most important prognostic factor. Examination of the fundus is warranted in all children with leucokoria and/or strabismus.

■ 1362

Management of uveal tumours

DAMATO B

Liverpool

Purpose The purpose of this presentation is to describe the management of uveal melanomas and the other most common uveal tumours.

Methods Choroidal melanomas are treated with plaque radiotherapy if possible, with proton beam radiotherapy, stereotactic radiotherapy, trans-scleral local resection, trans-retinal endoresection, phototherapy and enucleation being reserved for patients who cannot be managed with a plaque. Increasingly, tumour biopsy is performed for histological grading of malignancy and for cytogenetic studies aimed at determining the genomic tumour type so that risk of metastatic disease can be determined. Choroidal metastases usually respond to external beam radiotherapy. Biopsy may be needed to confirm the diagnosis. Choroidal haemangiomas are treated by photodynamic therapy, with good response in most patients.

Results In the large majority of patients, it is possible to conserve the eye and vision. Patients need life-long follow-up in case tumour recurrence occurs. After radiotherapy of choroidal and ciliary body melanomas some patients develop exudative and neovascular complications needing treatment.

Conclusion Successful management of uveal tumours is based on a firm diagnosis, accurate staging of disease, reliable prognostication and adequate aftercare.

■ 1364

Management of conjunctival tumours

MIDENA E

Padova

ABSTRACT NOT PROVIDED

■ 1365

Lid tumours

SEREGARDS
Stockholm

ABSTRACT NOT PROVIDED

■ 1366

Orbital tumours

PRAISE JU
Eye Pathology Institute, University of Copenhagen, Copenhagen

Purpose To give an overview of orbital tumours enabling the course participants to deal with patients suffering from such tumours.

Methods Schematic demonstration of the flow of clinical symptoms and tests. Discussion of the result of the tests.

At the end of the presentation a series of cases illustrate the key points of the lecture

Oral presentations

• Sessions on Thursday	30
• Sessions on Friday.....	83
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■ 2111

Segmentation of FD-OCT images shows selective loss of inner retinal layers in patients with DM and no or early DR

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Purpose Determine whether diabetes differentially affects specific retinal layers by comparing the thickness of six retinal layers in diabetic patients with no or minimal diabetic retinopathy (DR) to age- and gender-matched normal controls.

Methods Forty-four patients with type 1 diabetes and no or minimal DR underwent full ophthalmic examination, stereoscopic fundus photographs and spectral domain optical coherence tomography (OCT). Following automated segmentation of intraretinal layers of the OCT images, mean thickness was calculated for 6 individual layers of the retina in the fovea, the pericentral area and the peripheral area of the central macula and compared to an age- and gender-matched control group.

Results In type 1 diabetic patients with minimal DR, the retinal nerve fiber layer ($p=0.00$) and the ganglion cell/inner plexiform layer ($p=0.02$) were significantly thinner compared to age- and gender-matched controls. No other layers showed a significant difference.

Conclusion Thinning of the total retina in diabetic patients with minimal DR relative to normal controls is due to a selective thinning of inner retinal layers and supports the concept that early DR includes a neuro-degenerative component.

■ 2112

Assessing macular edema- prognostic parameters by OCT

DE SMET M

ABSTRACT NOT PROVIDED

■ 2113

Cirrus HD OCT algorithms for macula and retinal nerve fiber analysis

HORNE M

Dublin, CA

ABSTRACT NOT PROVIDED

■ 2114

High-definition Fourier domain OCT: non-invasive assessment of BRB changes

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Purpose To demonstrate the possibility of using a non-invasive imaging technique, the high-definition spectral domain optical coherence tomography, as a surrogate detector of blood-retinal barrier (BRB) breakdown.

Methods Healthy volunteers and diabetic patients with diabetic retinopathy, age-related macular degeneration, choroidal neo-vascularisation and cystoid macular edema, underwent optical coherence tomography by Cirrus HD-OCT (Carl Zeiss Meditec, Dublin, CA, USA). Profiles of reflectivity distribution between the inner-limiting membrane and the retinal pigment epithelium were built, normalized, aligned and compared. Patients underwent retinal leakage analyzer (RLA) to identify areas of retinal fluorescein leakage into the vitreous as areas of blood-retinal barrier breakdown.

Results The comparison between healthy volunteers' and patients' reflectivity distribution demonstrates these profiles differ. Moreover, when comparing reflectivity distribution within the same eye between areas of leakage and areas of non-leakage, the sum of the squared differences is over one decade relatively to the comparison between two similar areas (leakage/leakage or non-leakage/non-leakage).

Conclusion These findings suggest that high-definition OCT may be useful in identifying areas of retinal leakage/BRB breakdown and therefore to be used as surrogate for fluorescein angiography and retinal leakage analyzer.

■ 2121

Diabetic retinopathy - Apoptosis and BRB breakdown prevention in early diabetic retinopathy

DE SOLUZA-RAMALHO P (1), PEREIRA DA SILVA A (2), PEGO P (2), BICHO MP (2)

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(2) *Genetic Lab., Metabolic Centre, Lisbon University, Medical College, Lisbon*

Purpose Diabetic retinopathy (DR) is complex multifactorial neurovascular degenerative complication of systemic disease with evidence of inflammation/oxidative stress induced by hyperglycemia leading to early retinal cell apoptosis and BRB breakdown confirmed in STZ diabetic rats. Erythropoietin (EPO,40.3kDa) multifunctional protein approved drug for anemia has confirmed antiapoptotic effects in retinal cell cultures, various models of stress: ischaemia, reperfusion,glaucoma,diabetes: rats, mice, rabbits. It is upregulated in the retina in proliferative DR. We studied EPO serum levels in diabetics with and without retinopathy comparing to non diabetic controls.

Methods In 60 type 2 diabetics, 26 with and 34 without retinopathy of both sexes and age of 64.24 ± 11.64 years EPO serum was determined (mlu/ml) by ELISA and compared to 44 non diabetics, age and sex matched controls. Student t test was used.

Results EPO serum levels were high in retinopathy patients 15 ± 11.14 vs 9.47 ± 6.57 in controls $p=0.043$. Intermediary levels in diabetics without retinopathy 10.84 ± 6.11 .

Conclusion High EPO serum expression in diabetic retinopathy supports endogenous overproduction by Hif-1 α , as protective mechanism of BRB breakdown and vascular/neuronal cell apoptosis demonstrated in STZ diabetic rats. Intravitreal EPO prevented all early diabetic retinal changes by activation of Erk 1/2 pathway. Antioxidant/inflammatory related molecules earlier studied, TMR, Haptoglobin, ACP, MHR and EpOx give additional support to this hypothesis. These proteins are related to EPO and ACE activity. EPO besides retinopathy preventive is angiogenic with viscosity effects.EPO derivative carbamylated forms which do not raise hematocrit have been developed.

■ 2123

The effects of sub-conjunctival EPO administration on ERG and on the peripheral blood haematocrit in animal model (rabbit)

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(2) *Clinics Department*

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Purpose To assess the effects of subconjunctival EPO administration on retinal electrophysiology and on the peripheral blood haematocrit.

Methods New Zealand White rabbits (n=6) received 100 UI of EPO through the subconjunctival route. Blood for Complete Blood Count (CBC) was collected on day 0, on day 7 and on day 14 of the experimental protocol. Furthermore electroretinograms were performed on day 0 and on day 30 of the experiments.

Results Regarding CBC changes, the haematocrit values changed from $35.42 \pm 2.7\%$ on day 0 to $34.32 \pm 4.3\%$ ($p=0.390$) on day 7 and to $34.45 \pm 4.4\%$ on day 14 ($p=0.931$), showing no significant changes. Concerning the red blood cells (RBC $\times 10000/\mu\text{L}$) count, the values evolved from 6.02 ± 0.48 on day 0 to 5.65 ± 0.67 ($p=0.074$) on day 7 and to 5.67 ± 0.74 ($p=0.948$) on day 14, showing no significant alterations. On the contrary, on what regards the electroretinograms, although there were no significant changes on a-wave amplitudes, which evolved from $13.94 \pm 1.7 \mu\text{V}$ on day 0 to $13.67 \pm 0.8 \mu\text{V}$ on day 30 ($p=0.844$) and no significant differences on N1-P1 amplitudes which changed from $46.60 \pm 4.5 \mu\text{V}$ to $55.48 \pm 10.5 \mu\text{V}$ ($p=0.438$), there was a remarkable increase on b-wave amplitude of 49%, changing from $46.60 \pm 7.43 \mu\text{V}$ to $94.97 \pm 13.36 \mu\text{V}$ ($p=0.031$).

Conclusion On what concerns CBC profiles, subconjunctival EPO administration did not cause any significant changes on haematocrit or RBC values. Regarding electroretinography, there were no significant changes on a-wave or N1-P1 amplitudes, but there was a marked increase in the b-wave amplitude which tests for photoreceptor functionality, which might indicate a protective action against apoptosis of retinal photoreceptors even in physiological conditions.

■ 2122

The role of HIF-1 alfa in apoptosis and proliferative retinopathy

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Purpose In diabetic retinal capillaries, the earlier morphological changes include pericyte loss and acellular capillary formation. These processes are regulated by interactions among a number of pro- and antiangiogenic factors, including vascular endothelial growth factor (VEGF) and Angiopoietin-2 (Ang-2). We hypothesize that increased levels of methylglyoxal (MGO) in RPE cells disrupts the balance of VEGF/Ang-2 promoting endothelial cell death and vessel regression.

Methods Rats with moderate T2D, and retinal cell lines of epithelium (RPE) and endothelium (EC) were used. MGO levels were determined by HPLC. Immunohistochemical analysis was performed in retinas stained for VEGF and Ang-2. RPE cells were incubated with MGO in hypoxic conditions and the level of VEGF and Ang-2 was assessed by ELISA. EC were subsequently treated with the pre-conditioned media of the RPE cells. Cell death was determined by WB against Bax and Bcl-2, while EC proliferation was assessed by BrdU-incorporation and fibrin gel angiogenic assays.

Results Hyperglycemia increases the levels of MGO in retinas and RPE cells. MGO increases the levels of Ang-2 and strongly decreases the levels of VEGF in response to hypoxia. VEGF downregulation appears to result both from increased HIF-1 α degradation and low HIF-1 transcriptional activity. The MGO-induced imbalance in the VEGF/Ang-2 significantly increases the expression of Bax and decreases the levels of Bcl-2. Consistently, this imbalance leads to decreased proliferation of the EC.

Conclusion In diabetic retinopathy, accumulation of MGO may play a role in VEGF/Ang-2 imbalance, triggering the activation of the apoptotic cascade which induces decreased proliferation of retinal endothelial cells and as a consequence vessels regression

■ 2124

Tolerance and safety of ocular use of recombinant human erythropoietin (rhEPO). Neuroprotective effects in ocular hypertension/glaucoma

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(3) *Ophthalmology Department, Columbia University, New York*

Purpose The purpose of this study was to evaluate the long-term effects of monthly intravitreal injections of rhEPO in a rabbit model.

Methods Sixteen New Zealand rabbits were divided into 4 groups: control (no injection), saline injection, or rhEPO injections of 500 U and 1000 U (N=4 per group). The right eye of each animal was injected monthly over a period of 7 months. Fundus examination and electroretinography (ERG) were performed at 1 day prior, and 1 week, 1 month, and 6 months after the initial injection. After the final ERG, animals underwent fluorescein angiography and sacrifice one week later. Scotopic and photopic ERG amplitude and implicit times were analyzed by calculating a ratio between the right and the left eyes. Angiograms were graded for the presence of neovascularization or leakage. Statistical analysis was carried out using two-way ANOVA.

Results Fifteen animals were used for this experiment (1 developed a traumatic cataract and was excluded). Between all groups and time points, there were no statistically significant differences in the computed right eye:left eye ratios for the scotopic or photopic ERG components ($p>0.05$). No evidence of neovascularization or fluorescein leakage was seen on angiography. There were no visible differences in retinal architecture or thickness in the rhEPO groups when compared to uninjected controls.

Conclusion Monthly 0.1 ml intravitreal injections of rhEPO at a dose of up to 1000 U over 7 months is well-tolerated and does not cause adverse effects on retinal function, architecture, or vasculature in a rabbit model. A review of published data on rhEPO and Glaucoma will also be presented.

■ 2125

Intravitreal and plasmatic levels of erythropoietin after subconjunctival administration in rabbits

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(2) Ophthalmology, Genetic Laboratory, Metabolic Centre, University Medical College, Lisbon

Purpose Recently Erythropoietin (EPO) had been shown to have neuroprotective and neuroregenerative effects on retinal ganglion cells, apart from its erythropoietic properties, being a promissory alternative on ischemic retinal diseases. With the present study we pretend to evaluate the efficacy of subconjunctival injection for ocular EPO delivery.

Methods New Zealand albino rabbits (n=6) were used. Complete ophthalmic examinations were carried out before and after the injections for 15 days. The proceedings (intravitreal punch and subconjunctival injections) were carried out under general anaesthesia. Through the subconjunctival route 100 UI of EPO diluted in 50 µl saline solution 0.9% was administered. The opposite eyes of each animal served as controls. The vitreous and plasmatic concentration of EPO were measured using the ELISA method.

Results Administration of EPO through the subconjunctival route allowed a vitreous absorption that reached the highest 24 hours after administration with 0,6 mUI/ml of EPO quantified in 100 µl of vitreous sample. The systemic absorption reached the highest concentration 3 hours after subconjunctival administration and 48 hours after the administration the plasmatic concentration of EPO regained physiologic values. EPO was not detected on control eyes.

Conclusion All the previous studies used the systemic or intravitreal route of administration to acquire therapeutic concentrations of EPO on the retina, both difficult to use in clinical practice. In this study, the subconjunctival route proved to be a promising alternative for ocular EPO delivery. However further studies are necessary to assess the blood and intravitreal kinetics of EPO after subconjunctival administration.

■ 2131

The clinical potential of RPE cells in retinal repair

DA CRUZ L
London

ABSTRACT NOT PROVIDED

■ 2132

Stem cell applications for retinal pigment epithelial cell defects

SKOTTMAN H
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Purpose The experimental data on the retinal pigment epithelium (RPE) transplantation clearly show the potential of the cell therapy treatment strategy for RPE dysfunction. Human embryonic stem cells (hESC) may have a significant role as tools for drug discovery and for replacement of damaged cells. Human ESCs are potent origin for mass production of specific cells, thus providing unlimited opportunities for these strategies. However, the utilization of hESC-derived RPE cells in therapy requires an efficient and xeno-free differentiation protocol that follows good manufacturing practice (GMP) standards. In addition, it is essential to develop methods for reliable characterization of the cells to gain safe and clinically eligible cells.

Methods We have compared the differentiation potential of several hESC lines and induced RPE differentiation in the absence of animal feeder cells and bovine serum. The differentiation of cells has been followed by expression analysis of retinal cell markers by using qRT-PCR and protein analysis.

Results With our differentiation protocol, we were able to generate RPE cells from several hESC lines with satisfying efficiency. The typical pigmented cobblestone-like morphology and expression of retinal precursor markers were detected within one week. Further, RPE specific markers were up-regulated during the maturation of the cells.

Conclusion We have developed progressive differentiation protocol, which enables generation of RPE cells without animal feeder cells and serum induction. The generated cell material is of high quality and more compatible with GMP requirements than cells generated in previous studies. In my presentation, I will review some of the data using hESC for RPE production and our experience in utilizing the hESC-derived RPE cells.

■ 2133

Biomaterials for RPE tissue engineering and cell microencapsulation

UURTIA
Kuopio

ABSTRACT NOT PROVIDED

■ 2141

Regulation of retinal ganglion cell gene expression by bHLH transcription factors in the developing and ischemic retinas

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Purpose The loss of retinal ganglion cells (RGC) in the glaucomatous retina exhibits similarities to the pattern of neuronal degeneration detected after experimental ischemia. However, a short episode of retinal ischemia does not provoke damage but rather triggers an endogenous form of neuroprotection. HIFs are bHLH proteins that regulate hypoxic response in ischemic retinas and they are involved in neuroprotection. Hypoxic environments also occur in the developing embryo and create specific niches controlling cell differentiation. Genetic analyses of HIF functions have revealed the importance of oxygen as a key regulator of ontogeny. We have compared the transcriptomes of RGCs in ischemic versus developing retinas.

Methods Genome-wide screens were conducted to identify genes which are expressed in newborn RGCs and growing optic nerve axons and which are up- or down-regulated after venal occlusion by photodynamic thrombosis in the rat retinas.

Results Atoh7 is a bHLH protein which is central to the transcriptional network regulating the production of RGCs. Among the targets of Atoh7 there are genes involved in the general metabolism and energy supply – e.g., alpha-enolase (ENO1), glucose-6-phosphate isomerase (GPI). These glycolytic enzymes are also targets of HIFs and they are upregulated during hypoxia. To investigate the linkage of glycolysis and mitochondrial activity in RGCs, we monitored by confocal time-lapse imaging the dynamic distribution of mitochondria in the cell bodies and axons of RGCs that express HIF/Atoh7 targets in developing and ischemic retinas.

Conclusion Some gene expression programs involved in differentiating RGCs might be reinitiated in neuroprotection.

■ 2143

Role of inflammation-related gene polymorphisms in branch retinal vein occlusion

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Purpose Branch retinal vein occlusion (BRVO) is a common vision-threatening disease. Some cytokines have previously been shown to exert proatherogenic as well as prothrombotic effects. Gene polymorphisms affecting the expression of these cytokines are thus plausible candidates as risk factors for BRVO. The purpose of the present study was to investigate hypothesized associations between cytokine gene polymorphisms and the presence of BRVO.

Methods The present case-control study comprised 398 patients with BRVO and 355 control subjects. Using 5' exonuclease assays (TaqMan), genotypes of the following single nucleotide polymorphisms were determined: interleukin 1 beta (IL1B) -511C>T, interleukin 1 receptor antagonist (IL1RN) 1018T>C, interleukin 4 (IL4) -584C>T, interleukin 6 (IL6) -174G>C, interleukin 10 (IL10) -592C>A, interleukin 18 (IL18) 183A>G, tumor necrosis factor (TNF) -308G>A, monocyte chemoattractant protein 1 (CCL2) -2518A>G, interleukin 8 (IL8) -251A>T and RANTES (CCL5) -403G>A.

Results Neither genotype distributions nor allele frequencies of any of the investigated polymorphisms differed significantly between BRVO patients and control subjects (IL1B -511TT: 7.8% vs. 9.6%, p=0.68; IL1RN 1018CC: 12.1% vs. 13.5%, p=0.15, IL4 -584TT: 1.3% vs. 2.3%, p=0.58; IL6 -174CC: 17.8% vs. 18.6%, p=0.97; IL10 -592AA: 5.3% vs. 9.0, p=0.14; IL18 183GG: 3.0 vs. 6.2%, p=0.11; TNF -308AA: 1.5% vs. 1.4%, p=0.95; CCL2 -2518GG: 6.5% vs. 4.5%, p=0.48; IL8 -251TT: 26.9% vs. 28.7%, p=0.23; CCL5 -403AA: 3.3% vs. 4.5%, p=0.63).

Conclusion Our data suggest that none of the investigated cytokine gene polymorphisms is likely a major risk factor for BRVO.

■ 2142

The distribution of neuroglobin in mouse eye

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Purpose To determine the distribution of neuroglobin (Ngb) in mouse eye. Ngb is predominantly expressed in the nervous system, and at particularly high levels in the retina. Ngb may serve as a reactive oxygen scavenger and may protect the tissue of eye from ischemia/hypoxia injuries. However, the distribution of Ngb in the eye is still controversial.

Methods Two polyclonal antibodies against Ngb were used in this immunohistochemical study, both of which were raised in rabbits. One of these two antibodies was generated against the whole recombinant protein of mouse Ngb and the other was generated against amino acid positions 55-70 of mouse and human Ngb. The expression of Ngb was analyzed with light microscopy on tissue sections.

Results These two antibodies showed comparable results. Ngb was expressed in the layers of the retina, including the ganglion cell layer, inner and outer nuclear layers, inner and outer plexiform layers, the inner segments of the photoreceptors and the retinal pigment epithelium. Ngb was also detected in other structures of the eye, including the epithelium and endothelium of cornea, the stroma of iris, the ciliary body, the lens epithelium, and the sclera. However, Ngb was not expressed in the corneal stroma, the lens capsule, the lamellar fibers of lens, the pigment epithelium of ciliary body or the pigment layer of iris.

Conclusion Ngb was found widely distributed in mouse eye. This finding can be explained by the fact that most of the structures of the eye originated from neural crest/neural ectoderm. Future experiments will focus on the distribution of Ngb at the mRNA level (in situ hybridization), and the quantitative expression levels at baseline and after hypoxic/ischemic challenge.

■ 2144

Morphological and functional characteristics of the hyperautofluorescent parafoveal ring in retinitis pigmentosa

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Purpose To evaluate the morphology and function of the hyperautofluorescent parafoveal ring in patients with retinitis pigmentosa (RP) using optical coherence tomography (OCT) and microperimetry (MP).

Methods Twelve patients with RP who had hyperautofluorescent parafoveal ring were examined. In all patients, full ophthalmological examination, MP (Nidek Technologies MP1 Microperimeter), OCT (Topcon 3D OCT-1000) and fundus autofluorescence (AF) imaging (Heidelberg Engineering HRA) were performed. The results of OCT imaging and MP testing were superimposed on the AF image. We investigated whether the AF diameter correlated with photoreceptor inner segment/outer segment junction (IS/OS line).

Results On the site of hyperautofluorescent ring, the integrity of the photoreceptor layers was affected and the IS/OS line was absent. In the encircled area of normal AF, a preserved photoreceptor layer with an IS/OS line was present. A strong correlation ($r = 0.83$, $P < 0.0001$) between the horizontal diameter of the encircled area of normal AF and the length of the IS/OS line was seen. The mean retinal sensitivity was significantly lower ($P < 0.0001$) over the area of hyperautofluorescent ring (2.0 dB, CI 1.1 to 2.8) compared to the mean retinal sensitivity of the encircled area of normal AF (9.2 dB, CI 6.6 to 11.9). Outside the ring, the majority of patients seemed to have an absence of photoreceptor layer with no light perception, even if AF pattern appeared well preserved.

Conclusion The hyperautofluorescent ring demonstrates border of abnormal retinal morphology and function, which are only preserved within the area it encircles. It appears that the ring represents an area of intensive photoreceptor degradation.

■ 2145 / 240

Gene therapy mediates cone rescue and rejuvenation in the R91W mutant form of Rpe65-deficiency mice

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Purpose Given the advances of gene therapy studies to cure RPE65-derived Leber Congenital Amaurosis (LCA) (clinical trials phase I), it is of prime importance to examine how cones can be rescued in different mutant contexts. Consequently, we evaluated the effect on retinal activity and cone survival of lentivirus-mediated gene therapy in the R91W knock-in mouse model expressing the mutant Rpe65R91W gene.

Methods An HIV-1-derived lentiviral vector (LV) expressing either the GFP or the mouse Rpe65 cDNA under the control of a 0.8 kb fragment of the human Rpe65 promoter (R0.8) was produced. LV-R0.8-RPE65 or GFP was injected into 5-days-old (P5) or 1 month-old R91W mice. Functional and morphological retinal rescues were investigated at 4 months of age.

Results Increased light sensitivity was detected by ERG and pupillary light responses in animals injected with LV-R0.8-RPE65 at both P5 and 1 month compared to controls. Histological analysis showed improved expression of cone markers and cone outersegment morphology. Furthermore, the density of cones in the region of RPE65 delivery after treatment at P5 reached the wild type level. However, before injection at 1 month of age, only a fraction of the cones (40% of the number found in WT animals) in the Rpe65R91W/R91W mice expressed cone transducin, this fraction increased to 64% after treatment. Moreover, these cones appeared normal.

Conclusion We show that lentivirus-mediated Rpe65 gene transfer is very efficacious in early treatments and still efficient during the course of cone degeneration. Moreover, the treatment at 1 month shows a rejuvenation process of the diseased cones. Thus patient suffering from R91W mutation might benefit from a prolonged therapeutic window.

■ 2147 / 242

LOC387715/ARMS2 studies – gene sequencing as a procedure of choice

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Purpose To search for important mutations in LOC387715/ARMS2 gene.

Methods 80 patients with choroidal neovascularization subsequent to age-related macular degeneration undergoing anti-VEGF treatment were screened for LOC387715/ARMS2 mutations. PCR followed by gene sequencing was performed. If polymorphism A69S coexisted with R38STOP gene cloning with pGEM-T vector was done.

Results R38STOP mutation was found in 5 patients, A69S in 59 patients, in 3 cases R38STOP coexisted with A69S on the other allele. Due to substantial shortening of the R38STOP translation product, the protein is probably not effective. In patients with A69S polymorphism coexisting with R38STOP there is only A69S effective allele – so patients should be treated as A69S homozygous.

Conclusion There is increasing number of studies with A69S variant of ARMS2 but only gene sequencing provide reliable date in these cases. If R38STOP is taken into account, A69S is even more important AMD risk factor. Gene sequencing is advisable in studies with LOC387715/ARMS2.

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Importance of electroretinogram in bull's eye maculopathy

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Purpose To describe the retinographic, electroretinographic and ultra-structural alteration in a interesting family case of bull's eye maculopathy.

Methods A 14-year-old boy, his brother a 12-year-old boy and his sister a 10-year-old girl with visual loss, underwent complete ophthalmological exams, including retinography, electroretinography (ERG) and ultrastructural study by electron microscopy of the skeletal muscle, at the Clinical Hospital of the University of São Paulo.

Results All three children presented optic nerve pallor; arteriolar thinning and bull's eye maculopathy. The scotopic responses were absent or with low amplitude contrasting with normal flicker responses. Electron microscopy study detected the curvilinear bodies typical from Neuronal Ceroid Lipofucinosi (NCL).

Conclusion The initial diagnose of those children was cone-rod dystrophy. Diagnosis of NCL was established by normal ERG flicker and findings of characteristic electron microscopic curvilinear bodies. The electrophysiologic testing are very important in the early diagnosis of NCL.

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Hunter's syndrome and buphthalmos in a girl: an unusual ophthalmic association

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Purpose To report an unusual ophthalmic presentation of a case of Hunter's syndrome/ MPS II.

Methods A sixteen-year-old girl presented to us with total loss of vision and forward protrusion OU since early childhood. Detailed examination, including slit lamp biomicroscopy, Intra ocular pressure (IOP) and funduscopy was carried out. Thorough systemic evaluation including Computed Tomography (CT), metabolic and genetic analysis was undertaken in collaboration with internists.

Results Characteristic facies, detection of glycosaminoglycan (GAG) variants in urine (chondroitin sulfate B and heparin sulfate) and iduronate-2-sulphatase activity in fibroblasts/leucocytes confirmed the diagnosis of MPS II. Child had severe photophobia but with no perception of light OU. OU buphthalmos with Haab's striae was noted, making a clear view of the fundus difficult. IOP OU was elevated, and 90D slit lamp biomicroscopy revealed a total glaucomatous optic atrophy in both eyes. On CT there was thickening and edema of preseptal and periorbital soft tissue with marked thinning of the optic nerves with prominent perineural CSF sleeves, indicative of marked optic atrophy.

Conclusion Glaucoma is a known association of Hurler's, Scheie's and Maroteaux-Lamy syndromes but not Hunter's. In fact, there is only one report of suspected angle closure glaucoma in MPS II. Buphthalmos is not a likely presentation as the sclera in these patients is known to be thickened due to deposition of GAG. To the best of our knowledge, this is the first case report of buphthalmos in association with MPS II. The importance of a meticulous examination in this subset of patients cannot be overemphasised. An appropriate and timely intervention may result in a better quality of life for them.

■ 2151

Why do we need a biomechanical approach to the ocular rigidity concept?

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Ocular rigidity in ophthalmology is generally assumed to be a measurable surrogate parameter related to the biomechanical properties of the whole globe. Clinical tonometry and tonography, as well as recently developed methods to assess the ocular pulse amplitude and pulsatile ocular blood flow and measurements with the ocular response analyzer are based on the concept of ocular rigidity. Clinical concepts of ocular rigidity describe a resulting effect without considerations of possible diverse morphology and material properties of the different ocular tissues. It is commonly accepted that ocular rigidity is related to the elasticity of the sclera. Many formulations are however dependent on the internal volume of the globe, intraocular pressure, corneal biomechanics and thickness of the corneoscleral shell. Sometimes this is extended to biomechanical properties of the ocular vasculature and perfusion pressure. Therefore ocular rigidity is expressed in various units and has different physical meanings but the same name is used which is confusing. Ocular biomechanics introduces parameters of elasticity and viscoelasticity of the sclera, cornea and other tissues which consider the morphology of the different tissues describing their mechanical properties such as: Young's modules of the sclera and Poisson's ratios of the cornea. When applying these rigorous statements and methods of biomechanical modeling a unified concept for ocular rigidity can be developed in order to link the limited clinical concepts, to improve them and to better understand the results of clinical measurements.

■ 2153

Ocular rigidity in living human eyes in health and disease

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Purpose It is known that the balance between aqueous humor secretion and outflow rate controls the steady - state average intraocular pressure (IOP). It has been also early identified that blood circulation results to IOP fluctuations practically synchronous to cardiac function. IOP is the primary mechanical load to several ocular structures including the optical nerve head.

Methods In-vivo intraoperative measurement of these quantities as well as secretion/ outflow coefficients in humans allowed us to quantify ocular rigidity, IOP and its fluctuations (and pulsatile blood flow) in a series of physiological and pathological eyes. Ocular rigidity, outflow facility and pulsatile ocular blood flow were measured intraoperatively in a cohort of 63 patients undergoing cataract surgery. Measurements were also performed in a series of age related macular degeneration (AMD) patients.

Results The eye, is a living structure under a continuously varying mechanical load that is strongly related to ocular haemodynamics. Ocular rigidity ranged from 0.0122 to 0.0343 (mean 0.0208)µl-1. Outflow facility coefficient (derived from pressure decay curves) was 0.33 (sd 0.15)µl/min/mmHg. Pulsatile ocular blood flow exhibited a strong negative correlation to IOP in all subjects. The ocular rigidity coefficient was higher in wet AMD patients compared to patients with dry AMD and healthy controls.

Conclusion There are indications that this mechanical load, associated also to ocular rigidity, can not only inter-modulate blood flow but also have a long-term effect on other structures in the eye. Understanding the role of these parameters may contribute to the understanding of ocular disease.

■ 2152

Biomechanical models for clinical data analysis of pressure-volume relationship in the human eye

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Purpose To develop mechanical models describing the pressure-volume relationship for the human eye and to compare the results obtained with clinical data in order to find out which mechanical characteristics affect this relationship most significantly.

Methods The fibrous coat is treated as a connected elastic shell consisting of two spherical segments with different radii and mechanical characteristics. The dependence of the intraocular pressure on the volume is analyzed using three different models in which the sclera and the cornea are modeled (1) by soft shells; (2) by transversally isotropic shells with small tension modules in the transverse direction; and (3) by 3D elastic solids. The models are studied analytically and numerically, the latter using FEM ANSYS software.

Results The results are obtained over a wide range of parameters using all three models.

Conclusion The models proposed predict a generally nonlinear relationship between the intraocular pressure and volume. The parameters of both the sclera and the cornea affect this relationship, the role of the sclera being more important. In the first approximation the simple soft shell model is in good agreement with the clinical data.

■ 2154

Ocular rigidity and ocular response analyzer

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Purpose To study ocular rigidity and sclera crosslinking level at different stages of primary open angle glaucoma (POAG).

Methods Biomechanical parameters of the eye especially corneal hysteresis (CH, mm Hg) were measured in 238 patients (311 eyes) aged 40-84 (median age 67.4 yrs) at various stages of compensated primary open-angle glaucoma using Reichert Ocular Response Analyzer (ORA). Besides, scleral samples obtained during sinus trabeculectomy combined with sclera trephination in the inferio-exterior quadrant of 28 patients (28 eyes) with various stages of POAG were studied using differential scanning calorimetry (Mettler TA 4000 with DSC20 cell).

Results Average value (median) of CH gradually decreased from 10.1 mm Hg in the initial glaucoma stage (I) to 9.1 mm Hg in the developed (II) and 8.6 mm Hg in the advanced (III) glaucoma stage. The decrease of this clinical parameter is caused by structural and biochemical damage of the corneoscleral coat. In stage I, endothermic scleral collagen transition occurred at the median thermal peak $T_m=60.3$ grad.C, while in stages II and III the median peaks of scleral collagen melting emerge at higher temperatures: $T_m=62.0$ grad.C and $T_m=64.5$ grad.C, respectively ($p<0.05$). This testifies to a significant increase of scleral cross-linking and ocular rigidity during glaucoma development.

Conclusion Biomechanical and biochemical disorders of glaucomatous sclera may cause clinical changes of ocular rigidity of eyes with POAG. This may be an important link of POAG pathogenesis requiring special therapy.

■ 2155

Evidence for altered ocular rigidity in glaucoma

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Purpose Based on theoretical models and animal studies altered biomechanical properties of the optic nerve head and the sclera have been implicated in the pathophysiology of glaucoma. Only few data have, however, demonstrated such biomechanical alterations in vivo. We tested the hypothesis that patients with primary open angle glaucoma (POAG) have an abnormal structural stiffness based on measurements of intraocular pressure amplitude and ocular fundus pulsation amplitude.

Methods Seventy patients with POAG and 70 healthy control subjects matched for age, gender, intraocular pressure and systemic blood pressure were included in this study. The ocular pulse amplitude (PA) was assessed with pneumotonometry. The fundus pulsation amplitude (FPA) was measured using laser interferometry. Based on the Friedenwald equation a coefficient of structural stiffness (E1) was calculated relating PA to FPA.

Results Systemic blood pressure, intraocular pressure, and ocular perfusion pressure was comparable between glaucoma patients and healthy control subjects. FPA as well as PA was lower in patients with glaucoma than in healthy controls. The calculated factor E1 was significantly higher in patients with POAG (0.0454 ± 0.0085 a.u.) than in healthy control subjects (0.0427 ± 0.0058 a.u., $p = 0.03$).

Conclusion This study is indicative of increased structural stiffness of the sclera in patients with POAG. This is in agreement with a number of previous animal experiments and supports the idea that the biomechanical properties of ocular tissues play a role in the process of glaucomatous ONH damage.

■ 2156

Ocular pulse amplitude under pressure: what happens to OPA in glaucoma before and after surgery?

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Purpose To investigate whether trabeculectomy, besides its intraocular pressure (IOP) - lowering effect, has an effect on the ocular pulse amplitude (OPA). To determine if OPA changes are influenced by IOP changes.

Methods Forty-eight glaucoma patients (48 eyes) scheduled for unilateral first-time trabeculectomy were prospectively enrolled from October 2007 to April 2008. The eye undergoing trabeculectomy was considered as study eye, whereas the non-operated fellow eye was used as control eye. OPA, IOP, blood pressure and heart rate were measured prior to and 4 weeks following trabeculectomy by means of Pascal dynamic contour tonometry (DCT), Goldmann applanation tonometry (GAT) and sphygmomanometry. A regression model for repeated measures was used.

Results Preoperative GAT, DCT and OPA were 20.92 ± 8.55 mmHg, 21.33 ± 7.06 mmHg and 3.23 ± 1.58 mmHg, respectively. One month following trabeculectomy, GAT, DCT and OPA were 11.23 ± 5.03 mmHg, 14.45 ± 4.79 mmHg and 2.12 ± 1.07 mmHg, respectively. There was a significant decrease in OPA after filtering surgery in the study eye compared to the control eye ($P < 0.0001$). Changes in OPA were correlated with changes in IOP (Spearman $\rho = 0.49$, $P = 0.0004$). When the IOP change caused by filtering surgery was taken into account, no significant difference in effect on the OPA following trabeculectomy could be demonstrated in the study eye compared to the control eye ($P = 0.18$).

Conclusion OPA changes were strongly correlated with IOP changes. There was no evidence for an effect of filtering surgery on the OPA when the concomitant IOP decrease after trabeculectomy was taken into account.

■ 2161

Visual and cognitive function in adolescents born with very low birth weight - a 15- year follow-up

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Purpose To describe visual functions in adolescents with VLBW in comparison with a matched control group and to investigate associations with white matter damage of immaturity (WMDI), optic disc measurements and cognitive functions in the VLBW group.

Methods A total of 86 VLBW children survived the neonatal period during a 15 months period in the southeast region of Sweden. Fifty-nine of those, and 55 term control infants, participated in the 15-year follow-up study. Structural assessments included brain MRI, digital analysis of fundus photographs and cycloplegic refraction. Functional evaluations comprised best corrected visual acuity, stereo acuity, visual fields, ocular alignment, cognitive visual problems and intellectual level.

Results VLBW was significantly associated with smaller mean neural retinal rim area (in normal sized optic discs), subnormal visual acuity, subnormal stereo acuity and subnormal visual fields and increased frequency of astigmatism and ocular misalignment. Cognitive visual problems were more common and intelligence quotients were significantly lower in the VLBW group. WMDI occurred in 28% of the VLBW subjects. WMDI was significantly associated with visual field subnormality, myopia, ocular misalignment and cognitive visual problems. Subnormal results of performance subtests were associated with decreased visual and stereo acuity, ocular misalignment and cognitive visual problems.

Conclusion This study confirms previous observations that adolescents with VLBW are at a disadvantage regarding visual and cognitive outcome compared with adolescents with normal birth weight. Adolescents with WMDI had more pronounced visual and cognitive dysfunction.

■ 2163

Eye alignment in low birth weight and/or prematurity

ARINGE

neuroscience and physiology/ophthalmology, Göteborg

To describe eye alignment in children with low birth weight and/or born prematurely. Through a review of published studies and personal observations, describe what we have learnt from the past for the future about eye alignment. This talk will focus on what we know about eye alignment in different levels of prematurity and body weight as well as describe eye alignment in different patient groups with high frequency of low birth weight and/or prematurity.

■ 2162

Ocular motor development in normal and premature children

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The ocular motor system is immature at birth and the different types of eye movements develop at different stages of child development. This presentation aims to discuss the normal ocular motor development and the often delayed development of the ocular motor functions seen in premature children. For example, the visual fixation is often more unstable in the premature child. Also the saccadic as well as the smooth pursuit and vergence systems exhibit delays in development in the premature child. The delayed development has implications for the clinical investigation and must be taken into consideration when examining the visual functions of the premature child.

■ 2164

Visual impairment in children born before 25 gestational weeks - boys are more vulnerable than girls

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Purpose To describe the frequency of ROP and visual impairment with respect to gender in children born at gestational ages (GA) before 25 weeks.

Methods In a population-based group of children born at GA <25+0 1990-2002, who survived to 4 years of age (n=114), maximal ROP stages, treatment and visual outcome were followed up.

Results Almost all (97,4%) children had ROP and 63,2% were treated with retinal ablation. Visual impairment (VA<0.33) was more common in boys (32,6%) than in girls (9,2%). All visually impaired children but one had severe ROP as the primary cause of their visual impairment. Almost all visually impaired children had additional functional deficits.

Conclusion A large proportion of children born before 25 gestational weeks are visually impaired in spite of screening and treatment of ROP. Almost all visually impaired children in this group have additional functional deficits due to cerebral dysfunction.

■ 2165

Update on risk factors and future perspectives for preterm infants

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Purpose To give an update on risk factors for retinopathy of prematurity with special focus on postnatal growth and growth factors

Methods The relationship between birth weight, serum levels of IGF-I as well as postnatal longitudinal growth and ROP will be presented. Preventive measures will be discussed.

Results Birth weight data on 451 infants demonstrated initially a significant difference in BW between different ROP stages but when taking gestational age and sex into account the significance was eliminated. Recently, a new diagnostic tool based on weekly neonatal measurements of body weight and serum insulin-like growth factor 1 (IGF-I) levels, was shown to be predictive for ROP development. The algorithm "Weight IGF-I Neonatal ROP" (WINROP) predicted early (mean 10 weeks) all infants who later developed proliferative ROP requiring treatment. The WINROP algorithm was then taken one step further using only serial weight measurements (n=700), excluding blood sampling for measuring IGF-I. With this approach WINROP predicted all infants who later developed proliferative ROP requiring treatment (100% sensitivity) and correctly identified 75% of those who did not develop proliferative ROP, and thus would not need any ophthalmologic screening. We have also shown a close relationship between postnatal growth, severe ROP and poor brain development.

Conclusion For decades, neonatal intensive care has focused on survival of the most immature babies. Time has come to find methods to ameliorate the nutrition for the children born very preterm. It is known that IGF-I is essential for growth and development of the immature vasculature of the eye. Intervention with substitution of IGF-I to the very preterm babies to raise IGF-I up to normal intrauterine levels might be beneficial.

Commercial interest

■ 2171

A new tissue-polymer hybrid drug delivery system for artificial corneas

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Purpose We developed and evaluated a new hybrid tissue-polymer drug delivery system using lyophilized cornea and synthetic hydrogel. The feasibility of this system as a carrier and a drug delivery system for an artificial cornea (Boston Keratoprosthesis) was evaluated in vitro while non-modified cornea tissue was used as a control.

Methods Corneal tissue from the eye bank was first lyophilized. The tissue-polymer hybrid was synthesized by reconstituting the lyophilized cornea in a norfloxacin-loaded hydrogel solution followed by polymerization. Four different tissue-hydrogel compositions of varying hydrophobicity were synthesized and characterized over one month for the swelling and the drug release profile. Unmodified cornea tissue was used as a control. The mechanical strength and suture characteristics of the hybrid system and unmodified cornea were evaluated as a carrier for Boston K-pro using an artificial anterior chamber.

Results Both the hybrid-system and the control show excellent mechanical properties as carriers for the Boston K-pro. They withstood similar challenges of intrachamber pressures (50-70 mmHg) for wound stability. In vitro drug release analysis demonstrates a longer and more controlled drug release profile for the hybrid system as compared to the control. The most hydrophobic hybrid construct shows a release that is above the Minimum Inhibitory Concentration 90 of *Staphylococcus epidermidis* for the first two days.

Conclusion The new hybrid tissue-polymer system shows sufficient mechanical stability to serve as a carrier for the Boston K-pro. This system has the potential to simplify storage and distribution of donor tissue as a carrier for artificial cornea in developing countries where donor tissue is otherwise not readily available.

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Retinal detachments after Boston keratoprosthesis: incidence, predisposing factors and outcomes of repair

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Purpose Knowledge of predisposing factors to retinal detachments in certain patient populations can guide surgical planning in Boston keratoprosthesis placement.

Methods This is a retrospective, noncomparative, interventional case series. A review of 166 patients (203 eyes) who underwent Boston Keratoprosthesis implantation at the Massachusetts Eye and Ear Infirmary from 1993 to 2008 was performed. Most patients had very advanced ocular disease. Predisposing factors to retinal detachment and details of timing after surgery were investigated. Anatomical and visual outcomes of retinal detachment repair were analyzed.

Results

Table 1. Retinal detachment after K-Pro implantation

	Number of Eyes	Eyes with RD
Autoimmune systemic disease	72	24 (33%)
Chemical burns	14	3 (21%)
Non-autoimmune etiology	117	10 (8.5%)

Eighty-nine (89%) of patients with retinal detachments have visual outcomes of less than 20/200 despite surgical repair. Majority of retinal detachments in all three subgroups occurred within the first six months after K-Pro placement.

Conclusion These data suggest that a retinal detachment following K-Pro implantation is more frequent in patients with autoimmune disease (where K-Pro is generally not recommended). When they do occur, they portend a poor visual prognosis. Patients with underlying autoimmune systemic disorders should be warned of the higher risk of developing retinal detachments after keratoprosthetic surgery and warrant close co-management with a vitreoretinal specialist.

■ 2172

Evaluation of anterior chamber depth in aphakic and pseudophakic patients with Boston type 1 keratoprosthesis

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Purpose To assess anterior chamber depth in aphakic and pseudophakic patients with Type 1 Boston Keratoprosthesis using anterior segment optical coherence tomography.

Methods Sixteen patients who underwent Boston Type 1 Keratoprosthesis implantation were evaluated for anterior chamber depth by the non-contact technique of AS-OCT. Longitudinal cuts were used in evaluation of AC depth. Functional AC depth was defined as the distance between the back plate of the KPro and the anterior surface of the iris. Anatomic AC depth was defined as the distance from the posterior surface of donor cornea to the iris. Both functional and anatomic AC depths were measured nasally and temporally. The distance between the back plate of the KPro and anterior surface of the posterior chamber intraocular lens (PCIOL) was also measured in pseudophakic patients.

Results A total of 16 patients were included in this study. There were 9 pseudophakic and 7 aphakic patients. Anatomic AC depths ranged between 0.86mm to 3.2mm. Functional AC depths ranged from 0mm to 2.2mm. Pseudophakic patients had anatomic AC depths between 0.92mm and 3.1mm and functional AC depths between 0mm and 2.2mm. Aphakic patients had anatomic AC depths between 0.86mm and 3.2mm, and functional AC depths between 0mm and 2.2mm. The distance between the back plate of the KPro and PCIOL in pseudophakic patients ranged from 0.14mm to 2mm.

Conclusion Anterior chamber depth measurement, combined with AC angle data provide valuable information in the setting of KPro implantation. This may be helpful in monitoring patients post-operatively for anterior segment complications such as crowding of the AC, or occlusion of the angle.

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Prospective evaluation of the Boston keratoprosthesis

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Purpose To assess the sustainability of the Type I Boston Keratoprosthesis in the developing world and report 18-month follow-up data on the International Boston Keratoprosthesis Study Group pilot project in Ethiopian.

Methods Through an international collaboration between the Massachusetts Eye and Ear Infirmary, USA and the Menelick II Hospital, Ethiopia, six pre-selected patients underwent surgery with th Type I Boston Keratoprosthesis (KPro). Patient inclusion criteria included hospital access, multiple graft failure, or binocular blindness. An International Protocol was developed to standardize evaluation and follow-up. To reduce costs, the patient's own cornea was used as a carrier for the KPro when possible instead of a donor cornea. Follow-up reports were transmitted electronically to all physicians involved.

Results Six Ethiopian patients received a Boston KPro under local anesthesia without complications. In two patients, their own cornea was used as a carrier for the KPro. All patients had improved visual acuity from a baseline vision of hand motions. Postoperative vision ranged from CF at 5m to 20/40. All patients were able to self-administer their ophthalmic medications and five of six patients reported greater independence in ADLs. There were no infections, or compliance issues.

Conclusion Early results indicate that the Boston KPro may be an option in the developing world. KPro complication rates were no different from developed countries. The use of the patient's own cornea was not associated with a poor outcome. It remains unclear as to whether KPro may be superior to PK in this setting. Careful patient selection, standardized protocol implementation, and meticulous post-operative follow-up are necessary for long-term sustainability.

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Cost-effectiveness of the Boston keratoprosthesis

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Purpose Blindness due to corneal disease accounts for approximately 8 million of the 37 million blind in the world. A 2007 study confirmed the global cost-effectiveness (CE) of cataract surgery and penetrating keratoplasty (PK). We sought to determine the CE of the Boston Keratoprosthesis.

Methods In a retrospective chart review, patients who underwent KPro type I surgery in the United States over the last 5 years were identified. Patients had to have a minimum of 2 years of visual acuity follow-up data. Patients with autoimmune diseases and chemical burns were excluded. Eighty-three patients were included. CE was determined by cost-utility analysis (CUA). Complications and additional procedures were incorporated into a weighted cost decision tree. A conservative retention estimate of 85% was assumed at 5 years. Visual acuity values were converted to utilities and determined to be normally distributed per Shapiro-Wilk testing.

Results The mean preoperative utility value was 0.449 increasing to 0.630 postoperatively. The mean incremental utility was 0.182. Amongst our cohort, 65 patients experienced improvement in their BSCVA at 2 years, 13 experienced no improvement, and 5 deteriorated. A total discounted incremental QALY gain of 0.763 was obtained. The total discounted cost associated with this utility equaled \$11250 (USD). The cost-utility of the KPro was \$13106 per QALY. The model was assessed using both univariate and multivariate sensitivity analyses.

Conclusion The KPro is becoming an established procedure in the US for complex ophthalmic patients, often with numerous comorbidities. The cost effectiveness of KPro was determined in this study to be \$13106 USD per QALY. This is comparable to PK, which has been reported in the range of \$12000-16000 USD per QALY.

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Boston keratoprosthesis with titanium back plate

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Purpose Following implantation of a Boston Keratoprosthesis (BKPro), formation of a retroprosthetic membrane (RPM) is an indication of postoperative intraocular inflammation. The purpose of this retroactive study has been to compare BKPro back plates made of titanium with the standard PMMA back plates. Any difference in RPM formation would guide our future choice of material for the device.

Methods The design was a retroactive analysis looking at the surgical outcomes after 6 months in patients who had BKPro implants. Pediatric cases were excluded. The indications for having a KPro implant were broadly divided into three categories: autoimmune (n=12), chemical burns (n=11), and others (non-autoimmune) (n=55). Three types of back plates were studied:

1. threaded (screw-on) PMMA (n=39),
2. non-threaded (snap-on) PMMA (n=16) and
3. non-threaded titanium back plates (n=23).

Main outcome measured was incidence of RPM formation at 6 months after surgery that warranted removal by either YAG laser membranotomy or surgery.

Results The percentage of patients who developed RPM was greatest in the threaded PMMA group (46%), followed by the non-threaded PMMA (31%), although this difference did not achieve statistical significance (p=0.5). The non-threaded titanium group showed the least RPM formation (13%), which was significantly less than both the threaded PMMA (p=0.002) and the threadless PMMA (p=0.043) groups.

Conclusion Titanium KPro back plates induce less RPM than PMMA back plates, threaded or non-threaded. It seems that the titanium plates in this setting are more tissue friendly and cause less inflammation than PMMA.

■ 2211

Macular thickness alterations after cataract surgery determined by optical coherence tomography

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Purpose To evaluate macular thickness alterations with optical coherence tomography after phacoemulsification and posterior chamber intraocular lens implantation.

Methods In this prospective study, 201 patients who underwent phacoemulsification (102 men and 99 women) with mean age 65±8 years were included. Best corrected visual acuity, complete slit lamp examination with lens and OCT examination were performed in all patients before surgery and at one, three and six months postoperatively. Patients were divided into five groups: Group 1 (control group-100 eyes): patients without any predisposing factors for cystoid macular edema, Group 2 (15 eyes): patients with complicated surgery, Group 3 (27 eyes): patients with epiretinal membrane, Group 4 (35 eyes): patients with diabetes and Group 5 (24 eyes): patients with glaucoma.

Results The preoperative mean minimal foveal thickness (MMFT) in groups 1 and 2 was 204±24µm and 213±47µm respectively and had no significant changes throughout the follow up period ($p>0.05$). In groups 3, 4 and 5 a significant increase of macular thickness was detected. In group 3 the preoperative MMFT was 248±72µm and at 1, 3 and 6 months it was 263±86µm ($p=0.01$), 240±30µm ($p=0.18$) and 270±64µm ($p<0.01$) respectively. In group 4 the preoperative MMFT was 219±39µm, after 1 month the MMFT increased at 257±78µm ($p=0.002$), at 3 months it was 231±46µm ($p=0.005$) and at the last examination at 6 months it was 236±49µm ($p=0.005$). In group 5 the initially MMFT (206±21µm) had significant increase in the first [213±30µm ($p=0.07$)] and in the third month [223±24µm ($p=0.03$)].

Conclusion Diabetic retinopathy, epiretinal membranes and glaucoma may predispose to increase in macular thickness after cataract surgery.

■ 2213

Scanning beyond the limits of standard OCT: OCT scans of the peripheral retina and the anterior chamber angle with a slitlamp integrated FD-OCT system

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Purpose Exploring the quality of OCT images of the peripheral retina and anterior chamber angle made through a 3-mirror contact lens and a new FD-OCT device integrated into a slit lamp.

Methods Patients with peripheral lesions (n=10) and glaucoma (n=10), seen in the outpatient clinic of the Academic Medical Center, were scanned with a Fourier Domain OCT integrated into a common Topcon slitlamp (SLD light source, central wavelength 830 nm, bandwidth 30 nm, 1024 pixel CCD camera, scan speed 5k A-scans per second, up to 1024 A-scans per b-scan). For posterior segment scans a fast Z-tracking system in the reference arm compensates for the dynamic character (movements of patient, handheld lens, slitlamp) of the examination. Scans of peripheral lesions, and the anterior chamber angle were made with a 3-mirror lens, while simultaneously the lesions were observed with the slitlamp.

Results Scans of the peripheral retina obtained with a 3-mirror lens with the FD-OCT integrated into the slitlamp were of reasonably good quality and lesions, like peripheral laser scars, could be clearly identified. Compared to stand alone OCT systems, the integrated OCT system reached more peripheral lesions. The anterior chamber angle scanned through a 3-mirror lens enabled scans of the angle structures.

Conclusion It is possible to scan the peripheral retina and anterior chamber angle through a 3-mirror contact lens with the slitlamp with integrated OCT. These scans could be of clinical interest in patients with pathology in the peripheral retina pathology or the anterior chamber angle.

■ 2212

Slitlamp integrated OCT, what you can see, is what you can scan

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Purpose To present results of OCT images captured of the posterior and anterior segment of the eye using the SLSCAN-1, a new FD-OCT device integrated into a slit lamp.

Methods Patients, seen in the outpatient clinic of the Academic Medical Center, were scanned with the SLSCAN-1, a newly developed OCT scanning device, integrated into a common slit lamp (figure 1). The OCT is a Fourier Domain OCT system (SLD light source, central wavelength 830 nm, bandwidth 30 nm, 1024 pixel CCD camera, scan speed 5k A-scans per second, 1024 A-scans per B-scan). The posterior segment scans have been captured using a standard indirect ophthalmic lens (Volk). A color fundus photography of the observed area is made at the same time (Topcon camera DC1, resolution = 3.24 Mp).

Results For posterior segment imaging, the flexible optical arrangement of the slit lamp and the hand-held lens (Volk), allows the user to scan large areas of the retina (>45 degrees), depending on the pupil size of the patient. In all patients the macula and optic disk could be visualized without any difficulty. In the anterior segment mode, the SLSCAN-1 allows imaging of the cornea, anterior chamber, iris and parts of the lens.

Conclusion The images made by the SLSCAN-1, new slit lamp integrated FD-OCT, could be very useful to examine patients directly, both posterior and anterior, during regular slit lamp examinations.

Commercial interest

■ 2214

Screening for retinal detachment using a wide field scanning laser ophthalmoscope

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Purpose The development of non-mydratric retinal photography has changed the clinical practice, allowing detection of abnormalities in the posterior pole without clinical examination in mydriasis. However the field of view does not exceed 60° and peripheral retinal detachments are likely to be missed on these images. The purpose of this study was to evaluate a wide field (200°) imaging system (Optos, UK) using a scanning laser ophthalmoscope (SLO) for screening purposes in retinal detachment.

Methods All patients referred for retinal detachment from November 2007 to April 2008 were examined by one retinal surgeon who also performed the fundus drawing. An SLO image was taken by an orthoptist in training. A masked image lecture of the entire data base was performed by a resident. Both were unaware of the details of the retinal examination. The number of breaks and the extent of the detachment on the drawing were compared with the findings detected on the SLO image.

Results 56 eyes with retinal detachment were documented. In 40 out of 56 eyes the retinal breaks could be detected on the SLO images obtained. The retinal breaks situated superiorly between 11 and 1 o'clock or inferiorly between 5 and 7 o'clock and two retinal detachments, one superior and one inferior, were not detected on the SLO images. SLO image analysis enabled correct diagnosis of retinal breaks in more than 7/10 cases. Retinal detachments were detected in more than 9/10 cases.

Conclusion Although the wide field SLO imaging system is not suitable as a diagnostic tool of retinal breaks and retinal detachment replacing the fundus examination by a retinal specialist, it represents a reliable screening method.

Commercial interest

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Optical coherence tomography and biomicroscopical analysis in macular holes

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Purpose To assess the usefulness of optical coherence tomography (OCT) for better differential diagnosis of macular holes in comparison with biomicroscopical fundus analysis.

Methods We reviewed the files of 25 eyes of 24 patients who were diagnosed as having a macular hole on OCT examination and biomicroscopical fundus analysis. Each eye underwent six radial 3-mm OCT scans centered on the macula, one 6-mm vertical and one 6-mm horizontal scan. Retinal thickness was measured at the foveal center and 750 μm from the center, vertically, and horizontally. The diameter of the macular contour was also measured on vertical and horizontal scans.

Results On biomicroscopy only 8 eyes (32%) were diagnosed as having macular hole, while in the remaining 17 eyes (68%) diagnosis of macular hole was possible only performing an OCT examination.

Conclusion Optical coherence tomography is very useful in the diagnosis of macular holes compared with classic biomicroscopical analysis.

■ 2221

Does internal longitudinal microstructure of retinal veins change with age in medically healthy persons?

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Purpose We demonstrated previously that roughness of the retinal arterial blood column measured along the vessel axis, termed longitudinal arterial profile (LAP) increases significantly in anamnestically healthy volunteers with increasing age. Recently we have demonstrated age related changes in LAP of medically healthy persons. Whether longitudinal retinal venous profiles (LVP) are altered with age in this group is investigated.

Methods 83 medically healthy volunteers were examined by Dynamic Vessel Analyzer (IMEDOS, Jena, Germany) using stimulation with flickering light. 3 age groups were formed: young (N=28, 30,2±4,3 years), middle age (N=28, 42,3±3,3 years) and seniors (N=27, 64,0±5,0 years). Included in the analysis were volunteers without medical vascular risk factors defined as: blood pressure < 140/90 mmHg, HDL > 35 mg/dl, LDL < 190 mg/dl and glucose levels < 110 mg/dl. Retinal venous diameters were measured along 1 mm vessel segments to obtain LVP. Differences were analyzed using Fourier transformation.

Results In all age groups power spectra of LVP do not change during all stages of the venous response to the stimulation. There were no significant differences in LVP between the age groups. Venous diameters in middle-age group were reduced in comparison to the young group at all stages of vessel reaction (p<0,05).

Conclusion Our results indicate that in contrast to retinal arteries retinal veins of validated healthy volunteers do not undergo age related microstructural changes. Hence previously reported age related decrease of retinal venous diameter might have physiologic origin due to reduced blood flow in retinal microcirculation with age, not because of structural changes of venous wall.

■ 2223

Lornoxicam pharmacokinetics in the vitreous humor of albino rabbits

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Purpose To assess the elimination half-life of intravitreal lornoxicam, a non-steroidal anti-inflammatory drug (NSAID).

Methods Both eyes of 15 rabbits were intravitreally injected with 250 µg of commercially available lornoxicam (for intravenous/intramuscular use, Xefo® 8 IV/IM Injection, Nycomed Hellas S.A.). Six eyes were enucleated at time points 0, 1, 2, 6 and 24 hours after the injection was performed. The eyes were immediately frozen at -80°C. The vitreous was eviscerated from the eye and the drug was liquid-liquid extracted from a 0.4 ml sample. Lornoxicam was isolated by a reversed-phase High Performance Liquid Chromatography (HPLC) method at retention time 10.7 min and detected at 372 nm. The data were statistically analyzed in order to evaluate the pharmacokinetic parameters of the drug.

Results The recovery of lornoxicam after liquid-liquid extraction was calculated at 69.6% and the limit of determination was 0.1 µg/ml. Statistical analysis revealed that lornoxicam concentrations followed first-order kinetics with an elimination rate constant of 0.235h⁻¹ and a half-life of 3.0 h.

Conclusion The determination of the pharmacokinetic characteristics of intravitreal lornoxicam allows the possibility for further investigation of the drug's intraocular behaviour and therapeutic potential.

■ 2222

The AMD-associated complement factor H (CFH) polymorphism Y402H results in decreased CFH localisation to Bruch's membrane

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Purpose CFH down-regulates the alternative pathway of the complement system by binding to polyanionic structures on host cells/tissues and inactivating surface associated C3b. Recently, the Y402H polymorphism in CFH has been shown to be a major risk factor for AMD. Here we investigated the functional consequences of the Y402H polymorphism by testing the hypothesis that the resultant amino acid substitution alters CFH binding to macular tissue

Methods The 402H and 402Y forms of full-length CFH and recombinant CFH fragments (composed of CCP6-8) were labelled with different fluorophores (402Y with AlexaFluor-488 and 402H with AlexaFluor-594). These were simultaneously incubated with frozen sections of human macular tissue from donor eyes and the relative binding of the two forms was investigated. In some experiments the tissue sections were digested with glycosidic enzymes prior to incubation with the fluorescently-labelled proteins.

Results Whilst the 402H and 402Y variants showed similar levels of binding to the RPE, there was a marked reduction in binding of the 402H form to Bruch's membrane. The binding of both forms to Bruch's membrane was dependent upon interactions with heparan sulfates, and to a lesser extent dermatan sulfates.

Conclusion Complement mediated damage is important in the pathogenesis of AMD and the relative failure of the 402H form of CFH to localise to Bruch's membrane may result in over activation of the complement system at the retinal pigment epithelium/Bruch's membrane interface.

■ 2224

Subconjunctival injection of bevacizumab (Avastin®) for corneal neovascularization

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Purpose to study the effects of subconjunctival injection of Bevacizumab on corneal neovascularization.

Methods Prospective interventional case series on 7 eyes of 7 patients who underwent subconjunctival injection with Bevacizumab. The following parameters were studied pre-op, at 1 week, 30, 60 and 90 days post-op: UCVA, BCVA, pachymetry with OCT, slit lamp examination and photographic imaging. Conjunctival impression cytology pre-op at 1 week and 90 days was done and complications were also noted.

Results Pre-op diagnoses were: herpetic leucoma (4 eyes), chemical burn (2 eyes), neurotrophic keratopathy (1 eye). An informed off-label consent form prior to procedure was signed. 1.25 mg of subconjunctival Bevacizumab was injected nearest the area affected. Mean preoperative UCVA and BCVA were 0.86 and 0.44 LogMar units, improved to 0.61 and 0.26 LogMar units at 90 days post-op, respectively. Central and peripheral pachymetry improved from 532 and 623 microns pre-op, to 529 and 619 microns at 90 days post-op, respectively. All slit lamp findings and photographic imaging showed a clear regression of superficial and deep stromal corneal vascularization, with clearing of lipid deposits around the affected areas. No toxic effects were noted on conjunctival impression cytology.

Conclusion Subconjunctival injection of Bevacizumab is a safe and effective procedure for the regression of superficial and deep corneal neovascularization. It may be a good alternative for patients prior to performing an optical keratoplasty or for those who are poor candidates for the same.

■ 2225

Activity of corneal nociceptive nerve fibers during allergic challenge of the ocular surface

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Purpose The aim of this work was to study in vitro the spontaneous and stimulus-evoked electrical activity of corneal nociceptive nerve fibers during acute allergic inflammation of the ocular surface induced in the guinea-pig.

Methods Animals received i.p. 10% ovalbumin (OVA). 14 days later, a 10µl drop of OVA was applied topically to each eye. Blinking and scratching movements directed to the eye were measured during 10 min, and ocular symptoms (edema and hyperemia) and tear rate were measured. Animals were killed afterwards and both eyes were immediately excised and mounted in a superfused (32°C) recording chamber. Electrical activity of corneal sensory receptors was recorded from nerve filaments dissected from the ciliary nerves. Mechanical (calibrated von Frey hairs), thermal (bath solution temperature down to 20°C or up to 52°C), and chemical stimulation (30s-pulses of 98%CO₂) were performed. Spontaneous (SA) and stimulus-evoked activity were analyzed.

Results After the allergic challenge, eye-scratching behavior was present in 4 out of 15 animals and blinking movements increased from 1±0.05 to 26±5. Tearing also increased compared to control (33±3 vs. 5±1 mm). Compared to naive eyes, proportion of nociceptors with SA (17% vs. 5%) and spontaneous discharge rate (0.13±0.07 vs. 0.01±0.01 imp/s) were increased. Mechanical threshold of mechano-nociceptive units decreased significantly (0.37±0.05 vs. 0.89±0.13 mN). Chemosensitivity of polymodal nociceptors was slightly increased (1.87±0.42 vs. 1.34±0.23 imp/s).

Conclusion Changes in corneal sensory nerve activity observed acutely after allergic challenge of the eye surface may constitute the basis of itching and discomfort sensations, and hypersensitivity observed in allergic patients.

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Vasopressin receptors in ocular tissues and their impact on ocular hydrodynamics

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Purpose To investigate the effect of intravenous applied vasopressin on aqueous flow and to localize involved receptor types in ocular tissues.

Methods In anesthetized rabbits mean arterial pressure (MAP), intraocular pressure (IOP) and orbital venous pressure (OVP) were measured by direct cannulation of the central ear artery, the vitreous, and the orbital venous sinus, respectively. Laser Doppler flowmetry was used to record CilBF continuously. Aqueous flow (AF) was measured simultaneously by fluorophotometry. After baseline measurements arginine-vasopressin (AVP) was applied intravenously (infusion rate: 0.08 ng/kg/min). Immunostaining (SABC-method, immunofluorescence) was performed to localize potential vasopressin receptors in ocular tissues.

Results AVP caused a considerable increase of MAP (+6,90±1,21%; p < 0,001), a significant decrease of IOP (-9,56±2,35%; p = 0,003), a highly significant reduction of AF (-21,34±4,08%; p < 0,001) and no significant change of CilBF (-2,37±3,38%; n.s.). Immunofluorescent stainings show a characteristic pattern of V1- and V2-receptor distribution. Both, V1 and V2 receptors are detectable in choroidal, ciliary and iridal vessels. Moreover, they are localized in the ciliary epithelium, whereas the labeling is more intensive in the non-pigmented epithelium than in the pigmented epithelium.

Conclusion Although the applied dose of AVP did not changed CilBF considerably, a highly significant reduction of AF was observed. This suggests that the reduction of AF is predominantly caused by affecting the secretory mechanisms in the ciliary epithelium. The localization of vasopressin receptors in ciliary epithelium supports this assumption.

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Cytochrome C oxidase expression in retinal rod outer segment disks

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Purpose The rod outer segment (ROS) plays a pivotal role in the phototransduction cascade, a process which requires a high level of energy consumption. In rods ATP production is confined to the mitochondria in the inner part, and the distances between sites of energy production and expenditure do not seem adequate to supply phototransduction. The goal of this study was to test the hypothesis that Cytochrome c oxidase (Cox) is located and catalytically active in ROS disk membranes.

Methods Osmotically intact disks were isolated from 20 bovine retinal ROS obtained by sucrose gradient centrifugation in the presence of protease inhibitor cocktail and Ampicillin under dim red light at 4°C. Cox expression was studied by confocal laser scanning microscopy and semiquantitative Western-immunoblotting, with a specific antibody. Cox activity in disks was investigated spectrophotometrically.

Results Results from immunohistochemical confocal laser scanning microscopy, Western blot, and biochemical experiments suggest that Cox is uniformly distributed onto disks and therein catalytically active to an extent comparable to that of isolated mitochondria.

Conclusion The expression and activity of Cox in bovine ROS disks is suggestive of an aerobic metabolism in ROS. This study opens new scenarios on the energy production in rods and on the possible role of its deficiency in some important retinal diseases which have not been clarified yet.

■ 2231

The neuromuscular junction microenvironment in extraocular and limb muscles

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Purpose To characterise the components of the neuromuscular junction (NMJ) in normal and pathological extraocular muscles (EOMs) and to assess the dynamics of progressive denervation.

Methods Limb and EOM samples from 11 controls, 8 ALS patients and from transgenic mice with SOD1 mutations (D90A, G93A) paralleling familial ALS were processed for immunocytochemistry with antibodies against Schwann cell markers (S-100, p75, GFAP), gangliosides GD1b and GQ1b/GT1a, neurotrophic factors (BDNF, GDNF, NT-3, NT-4) and their receptors, parvalbumin, nestin, desmin and laminin chains.

Results The NMJs of normal EOMs had a different cytoskeleton composition. Differences in the expression of gangliosides GD1b and GQ1b/GT1a, Schwann cell marker S-100, agrin and nestin at the NMJs were noted in the human ALS EOMs. Parvalbumin was absent or scarce in EOM nerve trunks of ALS patients. The analysis of the time aspects of denervation in the animal models is ongoing.

Conclusion The human EOMs in late stages of ALS and the EOMs of the transgenic mice show signs of denervation, although these muscles appear remarkably well preserved. High levels of parvalbumin, proposed to be protective for oculomotor neurons in ALS, are not apparent in advanced stages of the disease. The identification of similar endpoints in the EOMs of patients with D90A mutation and the ALS transgenic mice carrying the same mutation indicates that this is a useful model to study the temporal aspects of progressive denervation in the EOMs, to explore aspects of muscle-nerve interplay that protect the EOMs in motoneuron disease and to gain further knowledge useful for the development of selective tools to modulate eye muscle function in the treatment of strabismus.

■ 2233

In search for correlation among markers for limbal stem cells niche

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Purpose The corneoscleral limbus is known to be the site of corneal epithelial stem cells (SC). Several molecules have been proposed as SC markers but none of them is able to univocally identify them. The aim of this study was to evaluate co-expression of different SC markers in human limbus.

Methods In this work five corneoscleral specimens from normal human donor eye-bank eyes (age 52-73 years) were fixed in formalin, divided in 8 segments, embedded in paraffin and examined by immunohistochemistry and immunofluorescence for p63, vimentin (vim), laminin 5, integrin (Int) $\alpha 6$, Int $\beta 1$, Int $\beta 4$, connexin 43, ki67 and N-cadherin positivity. We firstly analyzed the distribution and the anatomical structure of limbal crypts in each of the segments. Then we evaluated the percentage of positive areas in the niches. Finally we looked for colocalizations and possible correlations among markers.

Results We confirmed a different number of niches among the segments of the same corneoscleral rim. Moreover we observed high variability of niches number among patients which interestingly correlates with the percentage of p63 positivity of niche cells. Confocal microscopy double staining for p63 and vim did not show evident colocalization and vim+ cells were seen in the superficial layers rather than in the deep layer of crypts. Int $\beta 1$ staining directly correlated with p63 positivity while the remaining proteins appeared variably and widely distributed.

Conclusion Colocalization was evident at least for two SC markers (Int $\beta 1$ /p63) within the basal layers, while vim, expressed mainly in the superficial layers could act as late progenitor cell marker.

■ 2232

Keratinisation status and cytokeratins of the human Meibomian gland epithelium

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Purpose The Meibomian gland (MG) is an indispensable component of the functional anatomy of the ocular surface. Increasing evidence points to a high impact of hyperkeratinisation as a major cause of obstructive MG dysfunction (MGD) and evaporative dry eye. Information of normal keratinisation status and cytokeratin composition of the human MG is limited.

Methods Conjunctival whole-mount specimens including the lid margin from ten body donors of older age were embedded in paraffin. Serial sections were stained by H&E and Masson-Goldner stain and by immunohistochemistry with an antibody panel to cytokeratins.

Results In conventional stains, the MG shows distinct similarities with the pilosebaceous unit of the cilia. The keratinised skin epithelium extended into the terminal part of the MG excretory duct similar to the hair follicle. Preliminary IHC results showed that the epithelium was positive there for the skin keratin CK10. Along the central duct the keratinisation CK10 expression was gradually lost similar to keratinisation marker involucrin. However, filaggrin, a marker for incipient stages of keratinisation and located in keratohyalin granules continued in the superficial layer of the duct epithelium all along the Meibomian central ductal system. CK14 a marker for basal undifferentiated cells showed a homogenous expression all along the basal cell layer of the MG ducts and the acini.

Conclusion The MG shares similarities with the cilia in embryology, in structure and in the cytokeratin composition. It can hence be regarded as a "hair without a hair shaft". All parts of the MG ducts have signs of incipient keratinisation and preserve a commitment to keratinisation. Upregulation in MGD explain hyper-keratinisation as a typical event in obstructive MGD.

■ 2234

Chromosomal number aberrations and transformation in adult mouse retinal stem cells in vitro

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Purpose The therapeutic potential of stem cells on degenerative diseases and damaged tissues such as retinal degeneration has been recognized. Generation of high numbers of retinal stem cells (RSCs) in vitro would thus be beneficial for retinal transplantation. As long-term cultivated cells might be unstable and have a risk of transformation, it is important to assess the stability of these cells.

Methods We analyzed chromosomal aberrations of RSC lines isolated from adult and postnatal day 1 mouse retinas. Then, selected cell lines were tested for anchorage-dependent proliferation, and for possibility of transformation by transplantation in immunocompromised mice.

Results Aneuploidy occurred in all adult cell lines, albeit to different levels. Neonatal RSCs were the most stable and displaying a normal karyotype until at least passage 9. We observed that two of the adult RSC lines tested were transformed and identified several cell cycle proteins that might support the cell continuous proliferation and transformation.

Conclusion The aneuploidy level of adult RSCs did not necessarily correlate with cell transformation. Only the adult RSC lines passaged for longer period and with higher dilution ratio underwent transformation, showing that culture condition plays an important role in supporting the selection and growth of transformed cells.

■ 2235

Hypertonicity-induced decrease in aquaporin-4 expression in retinal pigmented epithelial cells

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Purpose Osmotic gradients regulate subretinal water content and might be acutely changed during macular oedema. Moreover, since RPE cells express tight junctions, water molecules must use specific channels to cross their hydrophobic membrane. Aquaporins (AQP) are good candidates to assume this function. In this work, we investigated the effects of osmotic stress on the expression of AQP in RPE cells.

Methods ARPE-19 cells were grown in different hypertonic conditions. AQP1 and AQP4 expressions were assessed by Western blot and RT-PCR. Chemical inhibitors were used to specifically block lysosomes and proteasome function. Cell proliferation was investigated by BRDU incorporation, and cell viability by flow cytometry. Cell cycle was studied by Western blot and flow cytometry.

Results Hypertonic stress rapidly decreased AQP4 expression on ARPE cells. The effect was reversed by proteasome inhibition, but was likely ubiquitinylation-independent. At 24h post-hypertonic stress, cell viability was not affected but cell proliferation was decreased. Cell cycle was also modified as the percentage of cells in G0/G1 phase decreased and the percentage of cells in S and G2/M phase increased.

Conclusion Hypertonic stress strongly reduced AQP4 expression and RPE cell proliferation. Those results might contribute to our understanding of macular oedema formation.

■ 2236

The Bmi1 polycomb gene as a target for therapies against retinal degeneration

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Purpose In several neurodegenerative diseases the reactivation of cell cycle proteins is a key event that precedes neuronal apoptosis. We asked whether a similar phenomenon occurs in Rd1 mice, a model of retinitis pigmentosa widely used to study photoreceptor (PR) loss.

Methods We used different knockout mouse models to reveal whether proteins involved in the cell cycle regulation are responsible for photoreceptor loss in the Rd1 mouse.

Results At P12, an early stage of the disease, Rd1 mice displayed an increased expression of CDK4 and CDK2 among PR nuclei. PRs also undergo DNA synthesis. At P12, the polycomb protein Bmi1 was expressed in virtually all the nuclei in the inner and outer nuclear layer of both wild-type (WT) and Rd1 mice. Bmi1 promotes cell cycle progression via the repression of tumor suppressor genes. We reasoned that Bmi1 deletion could impede the aberrant CDK reactivation that characterizes neuronal apoptosis and may therefore delay retinal degeneration. We compared the histology of WT, Rd1 and Rd1;Bmi1^{-/-} and observed the presence of 7 rows of PRs in Rd1;Bmi1^{-/-} mice at P33, while Rd1 littermates displayed a single scattered row of PRs. ERG recordings revealed the ability of Rd1;Bmi1^{-/-} retinas to respond to light stimuli. Both DNA synthesis and CDK4 were strongly decreased in Rd1;Bmi1^{-/-} mice, respectively by 70% and 50% as compared to Rd1 littermates.

Conclusion In conclusion, our data show for the first time a mechanism of retina degeneration involving a reactivation of the cell cycle that precedes PR death in Rd1 mice and reveal that the partial inhibition of cell cycle re-entry strongly delays PR loss.

■ 2241

Splicing defect in Calpain12 cDNA of a recessive cataract mutation in rats (cat)

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Purpose The recessive cataract mutation cat was identified among F2 offspring of Wistar rats having been treated with X irradiation; a corresponding mutant line was established (Léonard & Maisin, Nature 205, 1965, 615-6). Aim of the study is the identification of the underlying mutation.

Methods Homozygous cat rats have been outcrossed to wild-type Brown Norway rats; heterozygous F1 offspring (without cataracts) have been backcrossed to parental homozygous cat rats.

Results Among 461 F2 offspring, we observed in total 223 cataracts (48.5%) at the age of 10 weeks; however, 2/3 of these cataracts were discovered at the age of 3 weeks, and most of the remaining third at the age of 7-8 weeks. Linkage analysis located the mutation on rat chromosome 1 between the markers D1Got76 (76.2 MB) and D1Rat410 (87.6MB). This large interval contains candidate genes like Six5 and Opa3, which have been excluded by sequence analysis of cDNA from rat lenses. Recently, we identified a major splicing error in lens-derived cDNA of the gene Capn12 encoding Calpain-12. Besides exon 1, the transcript contains the complete intron 1; in exon 2 it keeps just a few bases to go to the end of intron 4 and than to exon 5; from there no further deviation from the wild-type sequence was yet observed. It is predicted that the sequence of the first intron leads to a premature stop codon after 10 new amino acids, which might result in a loss of Calpain-12 activity.

Conclusion Calpains has been proposed for a long time to be involved in cataractogenesis. Here we present for the first time a mutation in a calpain-encoding gene (Capn12), which is very likely to be responsible for cataract formation.

■ 2243

Phenotype-genotype relationship of TGFβ1 corneal dystrophies among Polish families

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Purpose To analyze clinical, histologic and genetic features of TGFβ1 corneal dystrophies.

Methods 43 patients from 14 unrelated families were recruited. The clinical examination consisted of inheritance pattern and medical history analysis, slit lamp biomicroscopy and anterior segment optical coherence tomography. Light microscopy was performed on 9 corneal specimen obtained during keratoplasty. PCR and sequencing of all coding exons of TGFβ1 was performed.

Results Based on the study we diagnosed 3 families with GCD1. The clinical pattern of all families with GCD1 was similar. OCT revealed hiperreflective focal changes in the stroma and distinct irregularity of Bowman layer. Histological analysis showed Masson trichrome positive deposits. R555W mutation was revealed. 1 family was diagnosed with TBCD. We noticed increased reflectivity, irregularity of Bowman layer and increased central corneal thickness (CCT) to 608 μm during OCT exam. R555Q mutation was revealed. Two mutations were detected in patients with LCD. Patients from 8 families had R124C mutation. Those patients had thin lattice lines in anterior stroma and CCT increased to 628 μm. Two families had atypical LCD with H626R mutation. There were differences in phenotype between those two families. The first one had asymmetric, late-onset dystrophy with thin fragile lines extending to DM on OCT scan, while the other family had both eyes involved with thick, distinct lines accompanied with stromal haze, also extended to DM. Corneal buttons of LCD patients showed Congo red positive deposits.

Conclusion Among Polish families R124C TGFβ1 mutation is the most common genetic pattern coexisting clinically with increased CCT. Genetic analysis plays an important role in corneal dystrophy diagnosis.

■ 2242

Genomic (epigenetic) DNA methylation in patients with open-angle glaucoma

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Purpose DNA methylation occurs by transfer of a methyl group to cytosine residues in the CpG1 islands. The extent of DNA methylation positively correlates with the extent of gene inactivation. The present study was performed to investigate whether there is an altered global DNA methylation in patients with open-angle glaucoma.

Methods This prospective case control study included 59 patients with primary open-angle glaucoma (POAG, age: 68 (SD 8) years), 54 patients with secondary open-angle glaucoma due to pseudoexfoliation syndrome (PEXG, age: 72 (SD 8) years), and 53 patients with cataract as controls (age: 69 (SD 11) years). Total DNA was extracted from frozen EDTA-blood using QIAmp DNA Blood Mini Kit (Qiagen). Global methylation status [DNA methylation in %, 1-(HpaII/MspI)] was measured with a modified non-radioactive assay. Statistics: Mann-Whitney-Test (2-tailed), the results are presented as means (SD), significance level p<0.05.

Results There was a significantly elevated genomic DNA methylation (in %) 1-(HpaII/MspI) in peripheral mononuclear cells in patients with POAG (68%, SD 18; U=868, Z = -2.79, p=0.005), but not in PEXG (55%, SD 17; U=1049, Z=-0.57, p=0.57) when compared with healthy controls (55%, SD 24). Thus, the difference between POAG and PEXG was exactly 13% of methylated HpaII/MspI restriction sites.

Conclusion Since methylation of DNA is an important epigenetic factor in regulation of gene expression these findings may have implications for a possible subsequent derangement of epigenetic control in patients with POAG. Further studies including gene-specific analyses are needed to clarify the differences between POAG and PEXG.

■ 2244

Gene screening at the 13q32 keratoconus locus

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Purpose Keratoconus (KTCN) is a non-inflammatory thinning and anterior protrusion of the cornea that results in steepening and distortion of the cornea, altered refractive powers, and altered visual acuity. We ascertained eighteen autosomal dominant multigenerational KTCN families from Ecuador and identified a novel locus on 13q32.1-q32.3 in the large family KTCN-014. Here we present sequencing results of candidate keratoconus genes localized to 13q32.

Methods The keratoconus locus contains 23 known transcripts. Sixteen of the genes were chosen for the evaluation. Genes are screened by standard techniques using 48 genomic DNA samples from individuals from family KTCN-014 and selected affected and unaffected individuals from other Ecuadorian families. Coding exons and intron-exon boundaries of the genes are evaluated.

Results Sequencing analysis of genes MBNL2, FARP1, ZIC5, ZIC2, FGF14, EFN2, RNF113B, DOCK9, PHGDHL1, VGCN1, ERG5 and ING1 have not revealed mutations segregated with the disease phenotype. Several novel single nucleotide polymorphisms were identified. Other candidate genes on 13q32.1-q32.3 including COL4A1 and COL4A2 are currently being screened for a possible role in the pathogenesis of KTCN.

Conclusion To date, mutation analyses of candidate genes have not identified sequence alterations segregating with the keratoconus phenotype in this population.

■ 2245

A novel congenital Stargardt-like macular dystrophy

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Purpose Many forms of macular dystrophies have been described including Stargardt disease, dominant Stargardt like disease, Best disease, Carolina macular dystrophy, Bietti's disease, and age related macular degeneration linked to ABCA4, CFH, C2 and ARMS 2 mutation or predisposing risk alleles. We describe a patient with a novel congenital macular dystrophy presenting with congenital nyctagmus and low vision.

Methods A 5 year-old girl presenting with low vision was seen for genetic counseling. She was born at term from consanguineous first cousin parents with nystagmus and corpus callosum agenesis. We report on her complete ophthalmologic neurologic and genetic findings.

Results Her visual examination showed distance acuity of 20/200 bilaterally and near acuity of P 4. Her fundus examination showed bilateral oval areas of macular atrophy. Angiography confirmed symmetric macular atrophy with Normal autofluorescence. Electrophysiology showed bilateral normal flash ERG and pattern VEP symmetric tracings showing no response to 15° pattern VEP at right and a delayed P100 response at left compatible with macular bundle dysfunction. OCT examination showed complete macular atrophy with thinning of the retina and interruption of the photoreceptor layer. ETDRS Center Macular thickness was 102 microns at right and 92 at left, ETDRS macular volume was 4.65 mm³ at right and 4.32 mm³ at left. ABCA4 testing was negative for mutations. Both parents had normal visual acuity, ERG, VEP and OCT and angiographs.

Conclusion This is the first presentation of a Stargardt congenital developmental macular dystrophy with apparently recessive inheritance.

■ 2246

Analysis of SERPING1 and its association with age-related macular degeneration

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Purpose The genetics of age-related macular degeneration (AMD) pathogenesis is a growing area of interest. In the last year a new potential association locus has been reported; SERPING1, a C1 inhibitor of the complement pathway. We present our own assessment of the SERPING1 AMD-risk locus.

Methods Patients with exudative AMD (n=94) and age-matched controls (n=95) were selected for study. All subjects were genotyped for the SERPING1 polymorphism identified as a new risk locus for AMD; rs2511989, IVS6-865 g>a. Genotyping was determined by direct sequencing. Statistical analysis was attained using SPSS v15 (SPSS Inc.).

Results Carriage of the G allele was raised in AMD group vs. controls (61.2% vs. 57.6%), but was not statistically significant (p=0.48). No significant associations were seen in genotype carriage between AMD and control groups (e.g. GG genotype, 40.4% vs. 30.4%, p=0.22).

Conclusion No significant associations were seen between SERPING1 and AMD in this study. Only two published analyses have examined this potential association, and these studies produced conflicting results. While it appears SERPING1 mRNA is produced in the eye, the functional effects of the rs2511989 locus have yet to be determined. More work is needed to identify the biological role, if any, SERPING1 plays in the development of AMD.

■ 2251

Until what age should glaucoma be monitored and/or treated? A novel index that facilitates clinical decision making

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Purpose To calculate for which combinations of age and perimetric disease stage (mean deviation [MD]) treated and untreated glaucoma patients are unlikely to become blind during lifetime.

Methods The probability of dying without blindness was calculated using the age and gender adjusted life expectancy values from the Statistics Netherlands and the progression rates of treated and untreated glaucoma patients from the Early Manifest Glaucoma Trial and the Groningen Longitudinal Glaucoma Study. Absence of blindness was defined as an MD better than -20 dB. Because it is difficult - or even impossible - to determine someone's individual life expectancy and rate of progression accurately, patients were assumed to reach the 90th percentile of the adjusted life expectancy and to progress with the 90th percentile of the rate of progression, leaving an a priori probability of becoming blind during lifetime of 2.5%.

Results If $MD+0.8 \times \text{age}$, with MD in dB and age in years, is larger than 59, treated male patients were unlikely to become blind during lifetime. For untreated male patients the cut-off value was 66, and for treated and untreated female patients 61 and 68 respectively.

Conclusion With this novel index, $MD+0.8 \times \text{age}$, the intensity of glaucoma monitoring and/or treatment can be reduced well-founded in the ageing patient. The index can be applied to either the worse or the better eye, depending on what is considered acceptable or possible given limited resources.

■ 2253

RTVue Fourier-domain OCT: reproducibility of RNFLT and macular thickness measurements

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Purpose To evaluate the reproducibility of peripapillary retinal nerve fiber layer thickness (RNFLT) and macular thickness (MT) measurements with the RTVue-100 Fourier-domain optical coherence tomography, and to determine the influence of pupil dilation, patients' experience in examinations and severity of glaucoma.

Methods One eye of 14 normal subjects, 11 patients with moderate, 12 patients with severe glaucoma and 40 screening trial participants were imaged 5 times on the same day. For the hospital-based patients, the measurement series was repeated after pupil dilation and 3 months later.

Results For the RNFLT and the MT parameters, intrasession intraclass correlation coefficient (ICC) varied between 93.9 and 99.0%, intrasession coefficient of variation (CV) between 1.95 and 5.69 %, and intratest variability between 3.11 and 9.13 μm . Most thickness values, all intrasession CV and intratest variability values and the signal strength index remained unchanged after pupil dilation. Most intrasession CV values increased significantly with increasing disease severity. Patients' experience in imaging examinations had no influence on intrasession CV. Intratest variability and intrasession CV represented 79.1 to 98.6 % and 77.1 to 95.0 % of test-retest variability and intervisit CV, respectively.

Conclusion Reproducibility of RNFLT and MT measurement with the RTVue-100 OCT are satisfactory for clinical purposes both in normals and glaucoma patients. Pupil dilation and patients' experience in imaging examinations do not influence the reproducibility of the measurements clinically significantly.

Commercial interest

■ 2252

Relationship between standard automated perimetry and high-resolution optical coherence tomography in glaucoma patients

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Purpose To determine the relationship between the main indices of standard automated perimetry (SAP) and the peripapillary retinal nerve fiber layer (RNFL) thickness measured with spectral-domain optical coherence tomography (OCT) in patients with glaucomatous visual field defects.

Methods 47 consecutive patients with open-angle glaucoma were included in the study. Only one eye per subject was randomly selected. SAPs were performed with a Humphrey perimeter and the 24-2 SITA standard algorithm. All of them underwent imaging with the Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany). Left eye data were converted to a right eye format. The Kolmogorov Smirnov test was applied to check that the data were normally distributed. Pearson correlations were calculated between SAP indices (mean deviation, pattern standard deviation, and visual field index) and OCT parameters

Results The average visual field mean deviation was -6.50 dB. Mild to moderate correlations were observed between SAP indices and most OCT parameters. The strongest correlations were found between the inferior quadrant thickness and pattern standard deviation (-0.544).

Conclusion The RNFL thicknesses measured with high-resolution OCT showed moderate correlations with SAP indices in glaucoma patients. These results may help to understand the relationship between structural and functional changes in open-angle glaucoma.

■ 2254

Retinal nerve fiber layer thickness and central corneal thickness in ocular hypertensive patients and healthy subjects

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Purpose To establish the correlation between central corneal thickness (CCT) and retinal nerve fiber layer (RNFL) thickness in ocular hypertensive patients and healthy subjects.

Methods We prospectively collected charts of healthy subjects and ocular hypertensive (OHT) patients in one academic center between 2007 and 2008. OHT patients were defined by two measurements of intraocular pressure superior to 21mmHg without treatment, open angle in gonioscopy, normal appearing optic nerve head and normal visual field test Standard Automated Perimetry (SAP SITA) and Frequency Doubling Technique (FDT). Every patient underwent a standard clinical examination including optic nerve head examination, intraocular pressure, CCT measurement by ultrasonic (US) and anterior segment OCT pachymetry, visual field testing (SAP and FDT), RNFL thickness by scanning laser polarimetry (GDX-VCC) and optical coherence tomography (OCT).

Results Eighty healthy subjects and 60 OHT patients were included. A correlation between US CCT and OCT CCT was found in both groups ($r_2=0.85$ and $r_2=0.87$, $p<0.001$). There was no significant difference ($p>0.15$) in GDX-VCC and OCT RNFL thickness in both groups. In controls there was no correlation of any RNFL thickness measurement with the CCT. In OHT patients, the US CCT was weakly correlated with the average TSNIT evaluated by GDX-VCC ($r_2=0.04$, $p<0.02$).

Conclusion This study did not show any relevant correlation between the RNFL thickness evaluated by GDX-VCC and the US CCT in healthy individuals and in OHT patients with a normal FDT.

■ 2255 / 220

Rarebit visual field follow-up in pediatric glaucoma

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Purpose To evaluate the long term change of the Rarebit (RB) visual fields and optic disc images in a group of children, maximally treated for paediatric glaucoma of various origin and severity, and compare them to normal RB visual field development during adolescence.

Methods Thirteen subjects (24 eyes) with pediatric glaucoma and 14 control subjects from the total group of 15 glaucoma and 15 control subjects in a previous study were included in the current follow-up study. For comparison, RB visual field data from 4 other studies including healthy children were used. Data regarding best corrected visual acuity, refraction (spherical equivalent), intra-ocular pressure, optic nerve appearance, diagnosis and treatment in the glaucoma group was collected from the medical records. In the control children, best corrected visual acuity was measured after determination of the refractive errors using an autorefractor. All participating subjects underwent RB visual field test and optic disc topography measurements using the Heidelberg Retina Tomograph.

Results A majority of both glaucoma eyes and control eyes showed visual field improvement. Using the same limit of normality as in the previous study (MHR above 90% and less than 5 depressed locations), 6 out of the 23 RB visual fields, previously classified as pathological, were now classified as normal. Some visual fields, both in the control and the glaucoma group showed slight deterioration, not leading to reclassification. Twenty-one of 26 eyes in the glaucoma group were examined with both RBP and HRT3. The concordance between the methods was 0.6 (Cohen's kappa).

Conclusion In adolescents, maximally treated for glaucoma, both the optic nerve heads and the visual fields remain stable during 5 years of follow-up.

■ 2256 / 221

The retinal nerve fiber layer and the optic nerve head morphology after glaucoma surgery

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Purpose To detect and quantify changes in the retinal nerve fiber layer (RNFL) and the optic nerve head (ONH) morphology after glaucoma surgery.

Methods 13 eyes of 13 patients with open-angle glaucoma in which goniotrephining with scleral flap without intraoperative antimetabolites for progressive glaucoma damage was done were included in this prospective study. Before and 6 months after the surgery: the intraocular pressure (IOP) was measured, the thickness of the RNFL was measured with a scanning laser polarimeter (GDx VCC), the confocal scanning laser ophthalmoscopy measurements of ONH with Heidelberg retina tomograph (HRT 3) were performed and the visual field was tested with Humphrey Field Analyser.

Results The mean IOP before surgery was 24.5 ± 2.3 mmHg decreasing 6 months after to a mean of 13.9 ± 3.0 mmHg ($p < 0.05$). The RNFL measurements with GDx VCC revealed no differences between the mean TSNIT Average ($p = 0.383$), mean Superior Average ($p = 0.756$) and mean Inferior Average ($p = 0.269$) before and after surgery. The ONH measurements with HRT 3 revealed postoperatively a significant increase in the mean Rim Area, Rim Volume and Cup Shape Measure, whereas Cup Area, Cup Volume and Linear Cup/Disc Ratio decreased ($p < 0.05$). There were no differences between the mean Height Variation Contour ($p = 0.678$) and Mean RNFL Thickness ($p = 0.064$) before and after surgery. Preoperatively the mean value of the Mean Deviation on automated perimetry was -18.82 ± 8.5 dB improving 6 months postoperatively to a mean of -16.63 ± 7.9 dB ($p < 0.05$).

Conclusion Our study demonstrated the beneficial effect of IOP reduction obtained with glaucoma surgery on visual field indices and ONH parameters evaluated by HRT 3.

■ 2261

Toxic effect of vigabatrin on retinal nerve fiber layer

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Purpose To investigate whether peripheral and central visual field defects detected among epilepsy patients treated with vigabatrin are associated with reduced peripapillary retinal nerve fiber layer thickness as measured with optic coherence tomography (OCT).

Methods Nine epilepsy patients with vigabatrin-attributed visual field loss (group 1) and seven patients (age and sex matched) with epilepsy treated with other drugs (control group 2) were regularly examined with automated perimetry up to 60 degrees from fixation point (Humphrey Field Analyser). Peripapillary retinal nerve fiber layer thickness (RNFLT) was quantified by optic coherence tomography (OCT) using Fast RNFLT protocol, Stratus OCT (3.0). Five of the patients in group 1 had peripheral visual field defects, (group 1a), four had a central field defect (group 1b). All patients in control group 2 had normal visual field.

Results Patients with vigabatrin-attributed visual field loss had attenuated total RNFLT compared to controls (right eye: mean total RNFLT: group 1: 75.6 μm (SD 12.7); group 2: 103.5 μm (SD 9.7), mean difference 27.9 μm (CI 15.9-39.9; $P < 0.001$). The nasal and inferior sectors RNFLT were more attenuated in patients with vigabatrin-attributed visual field loss compared to controls, while no difference was detected in the temporal RNFLT. Both individuals with peripheral and central visual field losses had attenuated mean total RNFLT compared to controls ($P = 0.006$ and $P = 0.002$, respectively).

Conclusion Vigabatrin-attributed visual field defects are associated with reduced RNFLT. Combination of perimetry and OCT can efficiently detect vigabatrin induced retinal nerve fiber damage.

■ 2263

Studying electrophysiological characteristics in children with congenital sensory nystagmus- case presentations

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Purpose In classification of sensory congenital nystagmus (CN) is important to recognize the underlying retinal or visual pathway dysfunction. The aim was to distinguish ERGs and VEPs characteristics which may identify among variety of disorders associated with sensory CN.

Methods In infants and small children that were ophthalmologically classified as sensory CN were ERGs and VEPs recorded simultaneously in the same session. ERGs were detected without dilated pupils and with skin electrodes. Under darkened laboratory conditions were ERGs recorded to white (cone/rod mediated response) and dim blue (rod mediated response) flash and under light room were ERGs recorded to white, red and 30 Hz flicker flash (cone mediated responses). VEPs were recorded from three occipital electrodes to flash and onset stimulation.

Results Cases with abnormal ERGs showed: in Leber's congenital amaurosis were undetectable both rod and cone mediated responses from early infancy; in cone-rod retinal dystrophy abnormal cone and rod mediated responses progressed in time; in achromatopsia abnormal cone mediated responses did not progress in time; in congenital stationary night blindness a negative ERG did not progress in time. Cases with abnormal VEPs showed: in ocular albinism VEP contralateral asymmetry; in achiasmia VEP ipsilateral asymmetry; in severe optic nerve hypoplasia flash VEP was non-recordable, while in moderate optic nerve hypoplasia flash and pattern onset VEP findings might not correlate with clinic findings.

Conclusion Sensory CN is associated with a variety of disorders affecting the retina, optic nerve, chiasm and electrophysiology may characterize retinal or postretinal pathway dysfunction and therefore help in early diagnosis.

■ 2262

Preterm and at term children: morphological and functional analysis of optic nerve and visual pathway with OCT, HRT and pVEP

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Purpose To compare optic nerve morphology and visual function in 14 preterm children (mean gestational age:33,38) with normal cerebral ultrasound at birth and 15 at term children (mean gestational age:39,67).

Methods Mean preterm age was 7,6 years, mean at term was 9,1 years. Children underwent to complete functional and morphological evaluation of Optic Nerve Head (ONH) with Heidelberg Retinal Tomography and Optical Coherence Tomography. The same children were evaluated at birth and at the time of morphological examinations by mean of transient and steady state pVEP.

Results Mean visual acuity was 9,85 in the preterm, 10 in the at term children.

HRT in preterm and at term children
at term (n=28) preterm (n=25) P value
mean sd mean sd

Disc Area 2,5 0,7 2,34 0,68 0,38

Cup Area 0,50 0,44 0,58 0,47 0,51

Rim Area 2 0,45 1,76 0,37 0,047

C/D Area Ratio 0,18 0,12 0,22 0,14 0,23

C/D Linear Ratio 0,4 0,15 0,45 0,16 0,30

Mean RNFL Thick 0,2 0,07 0,24 0,2 0,26

Tab.1

A statistically significant reduction was found in preterm children as regard rim area ($p=0,047$; tab 1) at HRT and superior RNFL thickness ($p=0,01$), temporal and inferior inner macular thickness ($p=0,03$) at OCT. Differences in the pVep latencies were found at 3 and 8 months after birth, but not at the time of morphological examination. Steady State pVEP amplitudes differences were not statistically significant.

Conclusion Morphologic optic disk and macula differences between term and preterm children seems not functionally affect the visual pathway.

■ 2264 / 341

Zone-I retinopathy of prematurity, progression and scheduling of treatment

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Purpose To evaluate the progression celerity and scheduling of suitable treatment time for Zone-I Retinopathy of Prematurity (ROP).

Methods Records of 36 eyes (18 infants) with Zone-I ROP, which were screened for ROP at the Neonatal Intensive Care Unit of Baskent University, Ankara, Turkey, between January 2004-March 2009, were evaluated retrospectively. Birth weight ranged between 480-1000g, gestational age ranged from 24-28 weeks. First fundus examination was performed at 29-31 weeks gestational age, and was repeated once or more per week. The first treatment was performed using laser photocoagulation and the progression criteria for laser photocoagulation treatment were:

(1.) Zone-I ROP less than stage-3 with plus disease (ETROP-type 1);

(2.) Zone-I stage 3 ROP with or without plus disease (ETROP-type 1).

Results Twenty eyes of 10 infants showed criterion (1) and 16 eyes of 8 infants criterion (2). Corneal opacity, pupillary rigidity, tunica vasculosa lentis and vitreous haze were observed until 31-33 weeks gestational age. The time period for the progression of stage-1 to stage-3 retinopathy ranged between 0,7-3,7 weeks. The mean age at the first treatment was 33 weeks (range 30-35 weeks). The mean time between the development of stage-1 retinopathy and the laser treatment was 9,8 days in mean (5-23 days), and 69,3 % of the infants were treated within 12 days after the diagnosis of stage-1 ROP. Additional treatments were performed in 7 eyes, scleral buckling+cryotherapy in 5 eyes, vitrectomy in 2 eyes. Thirty-two eyes had favorable and 4 eyes had unfavorable outcomes.

Conclusion The diagnosis of Zone-I ROP requires close-meshed follow-up and immediate treatment.

■ 2265

A new technique of anterior TAP enhances the positivity of CMV by PCR in hypertensives anterior uveitis

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Purpose Anterior uveitis can be severely disabling. Especially, hypertensives anterior uveitis can lead to a decrease in visual acuity, posterior synechiae, cataract, glaucoma, etc. Diagnosis is frequently complex. Two main aetiologies are retained: non infectious (auto-immunes) and infectious forms. Amongst the lasts, various aetiologies are possible. Viral anterior uveitis remained difficult to diagnose for a long time. However, since the emergence of the polymerase chain reaction (PCR), the diagnosis is definitely easier. Nevertheless, anterior TAP result is determined by different limitations including the puncture technique, the PCR primers used, and of course the investigated virus.

Methods We hereby propose a new technique of anterior TAP that allowed us to increase our PCR results in CMV anterior uveitis. Two samples were obtained: firstly, a conventional anterior TAP was realised; followed by a rinsing of the anterior chamber with saline solution. A Goldman-Witmer index for rubeola was performed in the first sample. Both samples were examined for viral PCR (HSV1, 2, VZV, CMV, EBV, Rubeola)

Results We did not found any side effect of the technique used by comparison with normal anterior TAP. Diagnosis was obtained in 20 of the 35 eyes tested. Rubeola diagnosis was obtained in 11/20 eyes, VZV in 1/20, HSV1 in 4/20, and CMV in 4/20. Intriguingly, CMV diagnosis was obtained in three cases only in the second syringe and not in the first

Conclusion We have, to date, detected 4 cases of CMV anterior uveitis in a cohort of 35 patients with anterior uveitis. We did not meet any complication but obtained interesting results concerning CMV diagnosis, using a rinsing of the anterior chamber (second syringe).

■ 2267 / 342

Foveal serous detachment in juvenile idiopathic arthritis(JIA)-associated uveitis

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Purpose To characterize the foveal serous detachment(FSD) in JIA-associated uveitis. To investigate the correlation with visual acuity (VA) and ocular inflammation.

Methods 9 children having FSD with JIA-associated uveitis were identified between 2005-2007. All were treated with periocular steroid injection and systemic anti-TNF α antibody. Outcome measures included VA, ocular inflammation quantified by laser flare photometry and the macular profile analyzed by OCT.

Results All patients (8 female, 1 male) had bilateral uveitis and 6 had bilateral SRD. All patients had risk factors to develop severe anterior uveitis. The mean age at the onset of uveitis and at the onset of FSD was 4.1 ± 1.1 years and 7.6 ± 2.2 years. At the onset of FSD 6 children were refractory to methotrexate and systemic corticosteroids. It had a high frequency of ocular complications: 87% posterior synechiae, 80% cataract, 60% band keratopathy and 20% glaucoma. FSD appeared isolated in 21% of eyes, it was associated with diffuse macular edema in 46% and with cystoid macular edema in 12% of cases. Before therapeutic intensification, the mean VA was $0.46 \log \text{MAR}$, the mean foveal thickness (FT) was $261 \mu\text{m}$. At 6 months follow-up: VA increased to $0.22 \log \text{MAR}$ ($p=0.017$), the reduction of flare was 41% ($p=0.003$), the mean FT was $229 \mu\text{m}$ ($p=0.59$). At 12 months follow-up, the mean VA was $0.19 \log \text{MAR}$ ($p=0.0029$), the mean FT was $196 \mu\text{m}$ ($p=0.009$), only 1 eye showed persistent SRD.

Conclusion FSD is a late-stage complication of sustained and insufficiently treated anterior uveitis in JIA-associated uveitis and must be considered for the long-term visual outcome. An aggressive immunomodulatory strategy is mandatory in order to achieve strict control of ocular inflammation and improve the visual function.

■ 2266

Efficacy of systemic ganciclovir as a therapeutic strategy for cytomegalovirus-associated anterior uveitis in immunocompetent patients

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Purpose Cytomegalovirus (CMV)-associated anterior uveitis is a newly recognized entity that accounts for half of patients with Posner-Schlossman syndrome. To date, the therapeutic management of these patients remains controversial. The aim of this study was to assess the efficacy of systemic Ganciclovir as a treatment for PCR-proven CMV-associated hypertensive anterior uveitis.

Methods Retrospective interventional study of 27 consecutive patients treated in a single centre between 2002 and 2008. Main outcome measures included intraocular pressure (IOP) and anterior chamber inflammation.

Results All patients responded to systemic Ganciclovir, with mean IOP dropping from 32 to 14 mmHg ($p<0.05$). Relapses occurred in 50% of cases within one year from the first antiviral course. Repeated treatment allowed control of the disease in 75% of cases (follow-up range: 12-78 months).

Conclusion Ganciclovir is effective but remains a suspensive therapeutic strategy.

■ 2271

Can the corneal endothelium of the pig proliferate in vivo?

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Purpose To further validate a recently developed pre-clinical model of corneal graft rejection in the inbred minipig, we have tested whether the corneal endothelium of the pig has proliferative capacity in situ.

Methods Eyes of pigs were enucleated at slaughter, corneas were removed aseptically and subjected to freezing injury. They were cultured in vitro in OPTI-MEM with 5% serum, endothelial side up, for up to 8 days. On days 0, 2, 4 or 8, corneas were washed, fixed and subjected to a silver stain to determine endothelial cell migration into the wound, or to Ki67 immuno-labelling to determine whether endothelial cells had entered the cell cycle. Corneas of transplanted minipigs were monitored post-rejection to determine whether graft opacity and oedema diminished.

Results By day 8 of in vitro culture there was up to 6-fold enlargement of endothelial cells on the periphery of the wound, but minimal migration of cells into the wound. Ki67+ endothelial cells were absent at harvest (n=5) and on day 1 of culture (n=3). Scattered isolated and occasional small clumps of Ki67+ cells were evident on day 2 (n=6), with no difference between injured and non-injured corneas. Such cells were interpreted to be detached epithelial or endothelial cells, because they were not associated with the wound margin, appeared to be above the cells in the endothelial monolayer and had smaller nuclei. Results to date of clinical post-graft monitoring reveal that corneal opacity and oedema did not improve by 37 days after maximal leukocyte infiltration, i.e. 50 days after onset of clinical rejection.

Conclusion The pig corneal endothelium appears to have minimal capacity to proliferate in vivo. This model would therefore be valuable for testing anti-rejection therapies.

■ 2273

A new storage medium for pre-cut donor tissue for DSAEK in eye bank

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Purpose To evaluate the efficacy of a new storage medium able to avoid swelling the donor cornea before the microkeratome cut in eye bank routine setting

Methods The Eusol-C (Alchimia s.r.l., Italy) storage medium and a modified storage medium (only for experimental use, Alchimia) were used to store two different sets of donor corneas (n=12). Before the microkeratome cut, corneas were stored in Eusol-C (n=6) while the contralateral donor corneas were stored in the modified medium (M.M.) (n=6). Donor lenticules were created with a full pass of microkeratome blade which resulted in a posterior lamella and a free cap. Endothelial cell density, cellular viability and corneal thickness by AS-OCT (VisanteTM, Carl Zeiss) were evaluated before dissection, 4, 24 and 48 hours after dissection.

Results Endothelial cell density and cellular viability were well preserved during the time course in both storage medium. Pachymetry values, after the microkeratome cut did not show significant variations during the time course. Conversely significant differences were found between the two groups (p<0.05). Average corneal thickness immediately before dissection was 659±38,08 microns and 537,83±24,78 microns in Eusol-C and in M.M. respectively. Immediately after dissection, thickness of the residual posterior bed was 247,83±44,13 (Eusol-C) and 143,6±24,96 (M.M.) microns. After 4, 24 and 48 hours was 238,5±41; 238,83±42,73 and 232,83±39,01 microns respectively in Eusol-C. Instead in M.M. after 4, 24 and 48 hours was 141,2±24,92; 145,6±22,35 and 157,2±30,40 microns respectively. The M.M. was able to reduce, on the contralateral corneas, the thickness of 100 microns in average.

Conclusion The M.M. was capable of preventing corneal tissue swelling during storage of pre-cut tissue for DSAEK.

■ 2272

Visual outcomes and safety of Intacs versus KeraRing intracorneal ring segment implantation in keratoconus

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Purpose To compare the safety and efficacy when two different types of intrastromal ring segments (ICRS): Intacs vs. KeraRing were used to treat keratoconus.

Methods Interventional, retrospective, comparative case serie study. A total of 53 keratoconic eyes were separated in two groups matched by age and degree of keratoconus: 31 (Group A) treated with Intacs ICRS and 22 (Group B) treated with KeraRing ICRS. Main outcomes were uncorrected, best corrected visual acuity (UVA and BCVA), sphere, astigmatism, spherical equivalent (EE) and mean keratometric values (km) pre and 3 month after implantation. Safety of the procedure was also evaluated. In both groups the femtosecond laser (IntraLase Corp, Irvine CA) were used to create the tunnel.

Results 3 month postop UVA were 0,26±0,2 vs 0,30±0,2 in Group A and B (p=0,6). BCVA was 0,61±0,2 and 0,67±0,2 in Group A and B (p=0,4). Postop sphere, cylinder and EE were -1,3±3,6D, 2,9±1,9D and -2,6±3,4D in Group A vs -1,3±1,8D, -3,1±2,0D and -2,2±2,4D in Group B respectively (p>0,05 in all comparison). Group A mean km was 43,1±12,6D vs 44,7±5,0D in Group B (p=0,6). Rate of explantation was 16,6% in Group A due to extrusion, neovascularization or non-controlled inflammation vs none in Group B.

Conclusion In this serie Intacs or KeraRing implantation to correct keratoconus seems to induce the same visual outcomes. However the tolerance of keraRing was better than intacs.

■ 2274

Effect of corneal collagen crosslinking on corneal sensitivity, tear function and innervation: a clinical and confocal microscopic studyKONTADAKIS GA, KYMIONIS G, PORTALIOU D, DIAKONIS V, PALLIKARIS IG
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Purpose To evaluate the effect of corneal collagen crosslinking (CXL) on corneal sensitivity, innervation and tear function.

Methods We conducted esthesiometry, Shirmer's II test, tear break up time measurements and confocal microscopy to evaluate innervation in 32 eyes with keratoconus preoperatively, and one, three and six months post CXL.

Results Sensitivity decreased significantly the first postoperative month (p<0.05) with restoration until the sixth. Tear break up time and Shirmer's II scores remained unaffected (p>0.05). Denervation appeared one month postoperatively and began retrieving by the third month.

Conclusion The main outcome of this study is that corneal innervation and sensitivity is significantly affected by crosslinking, but preoperative sensitivity is restored to the preoperative levels after six months.

■ 2275

Results of amniotic membrane transplantation with diathermocoagulation for corneal hydrops secondary to keratoconus

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Purpose To present the outcomes of corneal hydrops diathermocoagulation with amniotic membrane transplantation in eyes with keratoconus.

Methods Studied group consisted of 15 persons, 6 women and 9 men (mean age 31,14± 9,92 years). All patients had extensive and persistent hydrops in the course of keratoconus, present for more than 20 weeks and refractory to traditional therapy. Time of observation ranged from 1.5 to 3.5 years. Central corneal thickness (Visante OCT, Carl Zeiss), corneal protuberance, BSCVA, subjective eye's discomfort symptoms before and after the surgery, time to hydrops resolution and postoperative complications were assessed.

Results Hydrops was successfully resolved in all eyes. The mean time of hydrops resolution was 15,7±5,78 days. The mean central corneal thickness was 487,24±19,57 μm 4 weeks after surgery and 463,59±17,21 μm 8 weeks after surgery. BSCVA was 0,18±0,28 4 weeks after surgery (before surgery 0,042±0,27). Eye's discomfort symptoms (pain, tearing) collected 4 weeks after surgery completely receded in 5 patients (33,3%), decreased in 7 patients (46,6%), did not change in 3 patients (20%). Postoperative complications were: loss of contact lenses, suture loosening and corneal vascularization. In 3 patients, penetrating keratoplasty was performed with no episodes of rejection.

Conclusion Diathermocoagulation of hydrops provides faster complete recession of the edema. Amniotic membrane transplantation strongly and successfully reduces inflammation, scarring and vascularization of patient's cornea. This kind of combined therapy effectively treats persistent hydrops, increases the comfort of patient's life and stabilizes ocular condition before penetrating keratoplasty.

■ 2277 / 314

Comparison of deep anterior lamellar keratoplasty results in different TGFβ1 corneal dystrophies

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Purpose To compare the results of deep anterior lamellar keratoplasty in eyes with TGFβ1 corneal dystrophies.

Methods 10 eyes of 8 patients with TGFβ1 corneal dystrophies after DALK were recruited for the study. There were 5 eyes with LCD1 (R124C), 2 eyes with atypical LCD (H626R), 2 eyes with TBCD (R555Q), and 1 eye with GCD1 (R555W). Main outcome measures were: complication noticed during Descemet membrane air dissection, 6 month graft and stromal bed clarity, presence of stromal bed folds during OCT exam, best corrected visual acuity (BCVA), corneal astigmatism and endothelial cell density (ECD).

Results Local Descemet membrane perforation during DALK occurred in 1 eye with R555W mutation and 2 eyes with H626R mutation. Descemet membrane detachment was diagnosed postoperatively in one eye with R124C mutation. DMD resolved spontaneously within one month. At 6 month all grafts remained clear, but there were thick stromal bed folds visible during OCT exam in 1 eye with R555W mutation and 2 eyes with H626R mutation. BCVA was 0,8 in eyes with R555Q, 0,4 in eyes with R124C, 0,2 in eyes with H626R and 0,3 in eye with R555W mutation. There were no differences noticed in corneal astigmatism or ECD at 6 month. Mean corneal astigmatism was 3,3 D (± 0,43) and ECD was 2156 cells/mm² (± 758).

Conclusion The TGFβ1 dystrophy type influences the results after DALK. TBCD and LCD1 with no deep stromal or Descemet involvement are good candidates for DALK.

■ 2276

DSAEK grafted corneas remain clear with low endothelial cell counts

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Purpose Presentation of observations on endothelial viability and visual outcome in 30 consecutive eyes operated with Descemet's Stripping Automated Endothelial Keratoplasty (DSAEK)

Methods DSAEK for Fuchs' dystrophy was introduced in October 2006 at the Eye Dept. at Rigshospitalet. The first 7 eyes were grafted with pre-cut donor tissue, the remaining operations involved tissue preparation with a Moria keratome. All patients were monitored at regular post-operative intervals for an average of 20 months (range 7 - 34 months). Donor tissue condition was evaluated in terms of detachment rate, primary graft failure and rejection events. Endothelial cell counts (ECC) were done with a digital Topcon SP-3000P specular microscope camera. Final visual outcome was recorded.

Results A fairly large loss of endothelial cells during transplantation was noted. Average cell density of the donor buttons was 2400/mm². At one year follow-up the recorded ECC had declined to 1129/mm² on average. At two years the average cell density was 1083/mm² indicating a slow progressive cell loss. At the latest follow-up cell counts ranged from 429 to 2332/mm². Nonetheless all corneas were clear except for one, which was developing secondary bullous keratopathy. Patient satisfaction and visual performance and comfort did not seem correlated to the ECC. Median acuity was 20/40. No case of classical rejection was seen.

Conclusion DSAEK is a tempting alternative to PKP in Fuchs' dystrophy. The learning curve, however, is steep even for experienced surgeons at the cost of endothelial cells during the tissue handling. Corneal clarity as well as visual function is retained even with very low cell densities.

■ 2311

In vivo imaging of microglial cell trafficking

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Purpose Microglial cells (MCs) are active sensors of neural tissues that are rapidly mobilized upon disruption of homeostasis. Our goal was to observe in vivo the migration of MCs, which has not been done yet.

Methods Following acute laser damage, the behavior of MCs in the retina of adult Cx3cr1gfp/+ and gfp/gfp mice was observed noninvasively using time-lapse confocal scanning laser ophthalmoscopy. Observation were done at various time-points up to 8 days after laser damage.

Results Focal damage elicit prompt migratory response of MCs within 200 to 400 µm around laser burns. This migratory response was preceded in all case by dendritic reorientation. Convergent and nonconvergent migration were observed. Such migratory activity persisted several days after laser damage. At day 8, the microglia network was restored and microglial locomotion had ceased.

Conclusion To our knowledge, this is the first observation of microglial locomotion in vivo. A Morphological evidence of microglial activation starts with dendritic reorganization. Migrating cells were only of the dendritic type (i.e. not amoeboid). There appears to be a notable heterogeneity in the locomotor response of MCs. MCs within and around scars remain highly motile and mobile several days after laser damage.

■ 2313

In vivo confocal microscopic evaluation of inflammatory changes in the ocular surface

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Purpose The ocular surface constitutes a complex physiopathological and anatomical entity assuring the barrier between the outside world and the fragile ocular structures. Ophthalmic instruments such as the slit lamp, which magnifies approximately 40 times, cannot provide details of the corneal structures at the cellular level.

Methods In vivo confocal microscopy using the HRT Rostock Cornea module (HRT / RCM) provides better resolution and therefore outlines distinctively in vivo inflammatory changes occurring in the ocular surface.

Results In vivo confocal microscopy is capable of providing corneal, conjunctival and limbal cellular details in different ocular surface diseases such as dry eye, infectious keratitis, toxic keratitis, corneal intraepithelial neoplasia or vernal keratoconjunctivitis.

Conclusion In correlation with ex vivo impression cytology analysis, in vivo confocal microscopy constitutes an interesting aid in the diagnosis and management of complex ocular surface conditions.

■ 2312

In vivo imaging of retinal inflammation in experimental autoimmune uveoretinitis

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Purpose Experimental animal models are essential for us to understand the pathogenesis of human diseases. Posterior uveoretinitis can be modelled in mice with IRBP immunization (i.e. experimental autoimmune uveitis, EAU), whereas a number of mouse models are also available for age-related macular degeneration (AMD). With the advancement in new technologies, it is now possible to image inflammatory retinal changes in experimental mice in vivo non-invasively. The aim of the study is to clinical revisit the traditional retinal inflammation animal models with modern imaging techniques.

Methods EAU was induced in C57B/6 mice with IRBP peptide 1-20. Aged CCL2 knockout mice were used as an AMD model. Retinal inflammatory changes were imaged in vivo non-invasively using topical endoscopic fundus imaging system and the scanning laser ophthalmoscopy (SLO) system.

Results Inflammatory retinal changes in the early stages of EAU were characterised as retinal oedema, vascular sheathing, multiple small retinal infiltrates or large linear retinal infiltrates. "Snow-ball"-like vitreous infiltrates were observed in the inferior part of the fundus at the peak stage of EAU. Using SLO autofluorescent (AF)-macrophages were detected at the peak stages of EAU and were located predominately around inflamed retinal venules. At the late stages of EAU, retinal scars and intraretinal neovascular membranes were observed. In the retina aged CCL2 KO mice, regional retinal atrophy and dursen-like multiple lesions were observed. Dursen-like changes were autofluorescent in SLO examination. Ex vivo confocal microscopy indicated that they were not dursen but subretinal lipofuscin-loaded microglial cells.

Conclusion EAU mimics many aspects of human posterior uveoretinitis including retinal vasculitis, multifocal choroiditis. Late stage EAU could be a good model for inflammation induced retinal neovascularisation. CCL2 KO mouse is a model of dry-AMD.

■ 2321

Role of NO in retinal vascular disease

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Purpose Nitric oxide (NO) is a key regulator of vascular tone in all vascular beds including the eye. Hence, inhibition of NO synthase with L-arginine analogues leads to a reduction of blood flow to all ocular tissues. This enables the investigation of the role of NO in the physiology of blood flow regulation, but also abnormalities of the vascular L-arginine/NO system in ocular vascular disease.

Methods A variety of studies investigating the role of NO in healthy humans but also in patients with vascular disease is summarized.

Results Inhibition of NO synthase reduces retinal, choroidal and optic nerve head blood. A variety of studies also indicate that NO plays a role in the ocular vasodilator effects of numerous agonists including acetylcholine, bradykinin, carbon dioxide, histamine and insulin. In addition, NO appears to modulate the autoregulatory behavior of ocular vascular beds and is involved in retinal neurovascular coupling. In several ocular diseases such as diabetic retinopathy or open angle glaucoma abnormalities in the NO system can be observed.

Conclusion NO is a major regulator of ocular blood flow in humans. The existence of different NO synthase isoforms makes it, however, difficult to therapeutically intervene via the L-arginine/NO pathway. Further studies are required to characterize the role of the NO synthase isoforms in the control of ocular blood flow in more detail and to allow for therapeutic interventions in ischemic ocular eye disease via this attractive pathway.

■ 2323

Interventions via the NO system and tachyphylaxia

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ABSTRACT NOT PROVIDED

■ 2322

Introduction on the multifaceted roles of nitric oxide in the retina

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Multifaceted roles of nitric oxide in the retina. N.N. Osborne. Nuffield Lab of Ophthalmology, University of Oxford, Oxford, United Kingdom. Nitric oxide (NO), a free radical gas with a half-life of a few seconds is implicated in various physiological and pathophysiological roles associated with the retina and its vasculature. Generated by a family of nitric oxide synthetases (NOS), NO has been shown to bind to soluble guanylyl cyclase and to mitochondrial cytochrome c oxidase to activate defined signalling cascades. Different types of NOS exist and can be activated by calcium dependent (NOS1 and NOS3) or independent (NOS2) mechanisms. Generally, NOS1 is located to neurones while NOS2 and NOS3 are in glial and endothelial cells, respectively. NO is involved in communication between different neurones, glial cells and neurones, and in the interactions of endothelial cells with pericytes and neurones. As a consequence, a reduction in the generation of endogenous NO in the healthy retina can result in vasoconstriction; the consequences of such an affect on the retina and alterations in visual processing may alter the photoreceptor transduction mechanism and communication between retinal cells. The binding of NO to mitochondrial cytochrome c oxidase to effectively compete with oxygen has been suggested to be involved in a number of processes. NO-elicited events act as triggers by which mitochondrial signal transduction cascades become involved in the induction of cellular defence mechanisms and adaptive responses. Moreover, the effect of NO on the electron transport chain might lead to mitochondrial dysfunction and pathology. NO clearly has a multifaceted role in the healthy and unhealthy retina.

■ 2324

Nitric oxide and inflammation

DICK AD

Department of Cellular and Molecular Medicine, University of Bristol, Bristol

Purpose The talk will discuss the dichotomous role of nitric oxide in inflammation as a result of macrophage activation and also its role in controlling T cell responses.

Methods We have used both microglial cell cultures, bone marrow derived macrophages and finally animal models of uveitis to dissect the role of macrophage activation and nitric oxide production in both tissue damage and limiting the extent of the inflammatory response.

Results Macrophages when activated via T cell responses secreting interferon gamma, elicit a TNF-dependent nitrite response. Inhibiting nitric oxide activity by either suppressing NOS2 or via inhibiting TNF activity results in marked suppression of macrophage activation and reduction in retinal damage observed during experimental autoimmune uveoretinitis (EAU). Macrophages regulate T cell responses, in part via nitric oxide production, but is dependent upon IFN-gamma and autocrine TNF signalling via TNFReceptor1.

Conclusion TNF and Interferon play essential roles in generating macrophage activation that elaborates in turn nitric oxide production. The nitric oxide, whilst damaging to cell membranes thus contributing to tissue damage during autoimmunity, also assists in regulating T cell responses by down regulating of T cell proliferation within the target tissue.

■ 2325

Nitric oxide and cGMP protect the retina from ischemia and mediate somatostatin's neuroprotective effects

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Purpose The neuropeptide somatostatin has been shown to modulate retinal circuitry by activating its receptors (sst1-sst5) found in retinal neurons and to influence the levels of other neuroactive substances such as nitric oxide (NO) and cGMP. In addition, it displays neuroprotective properties against retinal chemical ischemia and excitotoxicity. In another paradigm, somatostatin was shown to protect cortical cultures against NMDA induced neuronal death via a cGMP mechanism. These findings led us to investigate whether NO and/or cGMP could protect the retina from ischemia, and possibly underlie somatostatin's neuroprotective actions.

Methods A model of chemical ischemia was employed in rat retina in order to examine the neuroprotective effects of arginine, the substrate of nitric oxide synthase (NOS), and a number of NO donors. Subsequently, blockade of NOS and guanylyl cyclase in the presence of somatostatin receptor (sst2) agonists was attempted to investigate the role of NO/cGMP in somatostatin's protection of the retina in the chemical ischemia model and in a model of AMPA induced excitotoxicity.

Results The NO donors SIN-1 and NONOate and 8-Br-cGMP protected the retina in a concentration-dependent manner, as shown by ChAT immunoreactivity and TUNEL staining. L-cysteine (the peroxynitrite scavenger) partially reduced the SIN-1 protective effect. NOS and guanyl cyclase inhibitors reversed the protective effect of sst2 agonists in the chemical ischemia and excitotoxicity model.

Conclusion NO/peroxynitrite and cGMP appear to be important mediators in the protection of the retina from chemical ischemia. The NO/sGC/cGMP pathway is involved in the neuroprotective effects of the sst2 ligands in the same model and against AMPA excitotoxic insults.

■ 2331

Anatomy of the Meibomian gland

KNOP E (1), KNOP N (2)

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 (2) *Dept. for Cell Biology in Anatomy, Hannover Medical School, Hannover*

Purpose The Meibomian gland (MG) is a large sebaceous gland in the eye lid that produces the lipids for the superficial tear film layer. Although it is an indispensable component of the functional anatomy of the ocular surface, its importance is still underestimated. Increasing evidence points to a high impact of MG dysfunction (MGD) as a major cause of a dysfunctional tear film and evaporative dry eye disease. Increasing new information has occurred in recent years and will be explained.

Methods Results of a Pubmed based literature review on the anatomy, cell biology and physiology are explained together with own results.

Results The Meibomian gland shares distinct similarities in embryology with the pilosebaceous unit of the cilia. The keratinised skin epithelium extends into the terminal part of the central duct. Signs of incipient keratinisation continue downstream in the central duct and ductules. The innervation of the MG is characterized by the structure of a dense network of nerve fibres and terminals around the basement membrane of the secretory acini and ducts. The MG is, similar to the lacrimal gland, mainly under parasympathetic control. The orifice of the central duct opens onto the free lid margin close to the posterior lid border and directly anterior to the line of Marx, that represents the surface of the muco-cutaneous junction which is followed proximally by the epithelial lip of the lid wiper

Conclusion The MG is the provider of the superficial lipid layer of the tear film and is regulated similar to the lacrimal gland which may allow coordinated secretion. It preserves a commitment to keratinisation which explains why hyper-keratinisation of the MG is an important cause for obstructive MGD. Deeper knowledge of the MG will allow better understanding of MGD and its therapy.

■ 2333

Keratinisation of the Meibomian gland as an important factor in Meibomian gland dysfunction (MGD)

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Purpose Obstructive Meibomian gland dysfunction (MGD) is a widespread alteration and represents a main reason for evaporative dry eye syndrome. Hyper-keratinisation of the ductal epithelium in response to various pathological stimuli appears as a typical underlying reason.

Methods Own morphological data together with findings from the literature on Meibomian gland anatomy, physiology and pathology are discussed and used to propose factors and their interactions involved in the pathology of obstructive MGD.

Results A large body of literature indicates that obstruction of the Meibomian gland is an important and widespread contributor to the pathology in MGD. Several findings indicate that keratinisation of the ductal system and orifice is the primary factor in obstruction of the gland. Histological and immunohistological data indicate that the whole ductal epithelium of the normal Meibomian gland shows signs of incipient stages of keratinisation, derived from its embryological ancestry. Hence, the gland preserves a commitment to full keratinisation if a proposed developmental block is removed due to various factors. Keratinisation leads to obstructive stasis of secretum inside the gland and to dilatation of the ductal system as well as to atrophy of the secretory acini. This results not only in decreased availability of Meibomian oil on the lid margin and tear film but also to secondary hypo-secretion. Increase of commensal bacteria and their products may have a certain role in this process although a real infection does not usually occur.

Conclusion Events in obstructive MGD which share hyper-keratinisation as a starting point, interact via different pathways that may form vicious circles and eventually lead to evaporative dry eye and gland atrophy.

■ 2332

Physiological impact of the Meibomian gland on ocular surface integrity

BRON A J

Nuffield Laboratory of Ophthalmology, University of Oxford, Oxford

Purpose To review Meibomian gland physiology, including the secretory process, role of the Meibomian lipids, their delivery to the tear film and the maintenance and stability of the tear film lipid layer.

Methods A Review

Results The Meibomian gland is innervated and possesses androgen and oestrogen receptors. The latter play a part in secretory regulation. Current evidence suggests that Meibomian oil is delivered in the absence of blinking but that delivery is aided by the blink. Only a proportion of the glands are active at any one time, which may reflect their holocrine nature; secretion involves total disgorgement of acinar contents. It may be envisioned that the glands go through a cycle in which some are active all of the time (maybe less than 50%) while all of the glands are inactive, some of the time. Studies of gland expressibility suggest that, for the lower lid, a far greater proportion of the nasal than temporal glands are active at any one time. Ninety percent of the meibomian oil consists of non-polar lipids while the rest is polar; phospholipid, thought to be essential for lipid spread across the aqueous tear film. Secretion maintains a basal level of about 300 mg of lipid in the oil reservoirs, of which about a tenth is delivered to the TFLL. Lipid is drawn across the tear film in the upstroke of each blink to form a lipid layer that stabilizes in about 1 second and remains stable for the remainder of the interblink. In the normal eye, the structure of the TFLL remains almost unchanged from blink to blink and then changes abruptly as the lipid from the TFLL mixes with that of the two oil reservoirs.

Conclusion These events ensure that the TFLL retards evaporative water loss from the tear film and conserves moisture at the surface of the eye.

Commercial interest

■ 2334

New insights into Meibomian gland function from gene expression experiments

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Purpose The definitive goal of scientific work is to elucidate connections and understand associated functions. In that respect comprehensive, expression studies using microarrays appear to be supreme instruments. We strived to reveal mechanisms of meibomian gland control by sex steroids.

Methods The gene expression of mouse meibomian gland under the influence of sex steroids is being presented based on own work group data along with results from literature work-up. GeneSifter Software is used for analysis based on z-score analysis and visualization of affected genes within known KEGG pathways.

Results Androgens were activating, for instance, pyruvate metabolism, steroid biosynthesis, regulation of autophagy, PPAR signaling pathway, lipid-related pathways but down-regulating immune-related pathways such as B and T cell receptor or Jak-STAT signaling pathways. Estrogens mainly influenced genes related to lipid metabolism and immune factors.

Conclusion The combination of gene expression microarrays and appropriate analysis software contributed significantly in starting to elucidate information on meibomian gland control.

■ 2335

Pathological alterations in Meibomian gland dysfunction as an important factor in ocular surface disease

BARABINO S

Ocular Surface Research Center, University of Genoa, Genoa

Purpose The purpose of this presentation is to synthesize a clinical description of Meibomian gland disease (MGD) diagnosis, classification, and treatments currently available and in development.

Methods The lipid content of Meibomian glands secretion varies with age and lid disease, and it contributes to the lipid component of the tear film by providing polar and nonpolar lipids. The lipids function as surfactant and barrier, provide structural support for the tear film, and are chiefly responsible for creating a smooth optical surface for the cornea. Meibomian gland disease (MGD) is one of the most common ocular disease characterized by an alteration of the lipids and therefore of all the ocular surface components, causing visual function changes and symptoms of discomfort.

Results The contribution of MGD to ocular surface disease includes contact-lens associated MGD, Meibomian keratoconjunctivitis, evaporative dry eye. The relationship between MGD and ocular surface inflammation will be presented with the results of clinical studies published in the literature to treat the different forms of MGD.

Conclusion MGD is a common but often misdiagnosed disease of the ocular surface which should be treated according to specific protocols.

■ 2341

The role of SERPING1 in age related macular degeneration

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(2) University of Cardiff, Cardiff

Purpose To evaluate the role of SERPING1 by genetic and functional analysis in a cohort of patients with Age related macular degeneration (AMD)

Methods We performed SNP genotyping to assess the role of SERPING1 in AMD. We also analysed other genes known to be associated with AMD and performed a variety of functional analyses. We developed a logistic regression analysis to predict the risk of AMD taking into account genetic and environmental risk factors.

Results Our logistic regression analysis accounts for 45 % of the risk of developing AMD. Results implicate the SERPING1 gene as being strongly associated with AMD. There is no convincing evidence for involvement of other genes or intergenic variants in LD with SERPING1 at this locus.

Conclusion In our cohort SERPING1 is a strong genetic risk factor for AMD. Ongoing functional analyses of SERPING1 will be presented at this meeting.

■ 2343

Genetic influences on biomarkers in AMD

CHONG V

Oxford Eye Hospital, Oxford

Purpose To describe the genetic influences on biomarkers in age-related macular degeneration (AMD).

Methods We have previously demonstrated that AMD is associated with systemic activation of complement factors, alternation of cytokine levels, and changes in the matrix protein level. In this study, we examine the association of these changes with the genotype of the common "AMD" genes.

Results Activation of complement factors were closely associated with "at risk" complement factor polymorphisms. Plasma cytokine levels were not associated with complement factor H polymorphisms, but were associated with the "at risk" HTRA1 polymorphisms. Plasma matrix protein showed a trend association with the "at risk" HTRA1 polymorphisms.

Conclusion Traditional genotype-phenotype correlation is based on clinical phenotype. Biomarker research is leading to a new area of genotype-phenotype correlation research. It is unclear at this stage, how important these correlations are. Further studies are required.

■ 2342

CFH, C3 and ARMS2 are significant risk loci for age-related macular degeneration but not for late stage disease progression

SCHOLL HPN

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Purpose Variants in the genes encoding complement factor H (CFH), complement component 3 (C3) and age-related maculopathy susceptibility 2 (ARMS2) have repeatedly been shown to confer significant risks for age-related macular degeneration (AMD). However, their role in disease progression and thus their relevance for the design of targeted therapeutic intervention remains unknown.

Methods Association between variants in CFH, C3 and ARMS2 and disease progression of late atrophic AMD was analyzed. Patients were selected from the multicenter FAM study cohort (n=619) and compared with 612 matched controls. A quantitative phenotype of disease progression was computed based on longitudinal observations by fundus autofluorescence imaging.

Results In a subset of 99 cases with pure bilateral geographic atrophy (GA), variants in CFH (Y402H), C3 (R102G), and ARMS2 (A69S) are associated with disease ($P=1.6 \times 10^{-9}$, 3.2×10^{-3} , and $P=2.6 \times 10^{-12}$, respectively). Median progression rate of GA over a mean follow-up period of 3.0 years was 1.61 mm²/year with high concordance between fellow eyes. No association between the quantitative progression variable and any of the genetic risk variants at the three loci was observed ($P>0.13$).

Conclusion Variants at CFH, C3, and ARMS2 confer significant risks for atrophic AMD; however there is no association with disease progression. Consequently, treatment options targeted at ameliorating pathogenic effects mediated by any one of the three susceptibility factors are likely to fail once atrophic manifestations of AMD have developed. Other, as yet unknown susceptibilities may influence disease progression.

■ 2344

Functional genomics of systemic complement activation in AMD

EDWARDS AO

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Purpose To describe the current status of genetic variants associated with age-related macular degeneration (AMD) and present functional genomic association studies demonstrating that systemic complement activation is influenced by haplotypes in AMD risk loci and increases the risk of having age-related macular degeneration.

Methods A genome wide association study using the Illumina 370 Beadchip on over 3000 subjects was performed. Focused genetic association and haplotype studies investigating the genetic mechanisms increasing AMD risk in complement and non-complement AMD risk loci were performed using standard methods. Functional genomic association studies relating genetic risks for AMD to plasma complement levels measured using ELISA assays were performed.

Results The collaborative genome-wide association study revealed novel pathways increasing the risk of AMD and will be discussed. The established genetic variants in the CFH, CFHR, FI, ARMS2 (10q26), and ApoE loci will be reviewed with a focus on insights from genetic studies on functional consequences. The proteogenomic association study showed that haplotypes in complement genes associated with AMD altered plasma levels of complement substrates, regulators, and activation by-products. The majority of the difference between case and control levels of complement proteins in plasma was explained by the genetic variants associated with AMD.

Conclusion Multiple independent pathways leading to AMD have been identified using the genetic approach. Detailed genetic studies and functional genomic studies are beginning to suggest mechanisms through which AMD risk is increased.

Commercial interest

■ **2345**

Can diet reduce the genetic risk of AMD?

*KLAVER C
Rotterdam*

ABSTRACT NOT PROVIDED

■ **2346**

Individualised risk in AMD

*MACKEY D
University of Western Australia, Lions Eye Institute, Nedlands*

With the advent of direct to consumer DNA testing, several companies are offering testing to patients for genetic risk of AMD. This testing is provided with significant information about AMD and its genetics. However, patients and their families will ask their ophthalmologists about this and ophthalmologists will need to be aware of what is being provided.

■ 2351

Ocular blood flow autoregulation and the clinical implications of its alteration

GARHOFER G

Department of Clinical Pharmacology, Vienna

Autoregulation is commonly defined as the ability of a vascular bed to adapt blood flow to changes in ocular perfusion pressure (pressure autoregulation) or to adapt to changes in metabolic need (metabolic autoregulation). Considering the high metabolic turnover of the eye, its intact function is strongly dependent on a stable blood supply, assured by an intact vascular autoregulation. However, it has been shown that in the recent years that several ocular diseases such as glaucoma, diabetic retinopathy or age related macula degeneration are associated with an impaired autoregulation. This vascular dysregulation may lead to an under- or overperfusion of the tissue and in turn to ischemia and/or oxidative stress. This talk seeks to summarize our current knowledge of autoregulation in the ocular vascular beds. Furthermore, the possible reasons of impaired autoregulation and how this may relate to ocular pathologies will be discussed.

■ 2353

Disease mechanisms leading to impaired blood flow in glaucoma

GHERGHEL D

Vascular Imaging and Research Laboratory, Aston University, Birmingham

Purpose SIS lecture

Methods Literature search

Results Although primary open-angle glaucoma (POAG), is associated more closely with elevated intraocular pressure (IOP), other risk factors already implicated in the aetiology of this disease and especially in the aetiology of normal-tension glaucoma are: abnormal ocular circulation, ocular and systemic vascular dysregulation, as well as systemic blood pressure (BP) alterations. Oxidative stress, which occurs as a result of an imbalance between generation of reactive oxygen species (ROS) and antioxidant defence mechanisms and is implicated in the pathogenesis of disorders ranging from atherosclerosis to neurodegenerative disorders, diabetes and aging, may also contribute to the general vascular disturbances observed in glaucoma. Moreover, increasing evidence shows that oxidative stress plays a role in promoting endothelial dysfunction, which is a key factor in progression of vascular diseases. Indeed, glaucomatous optic nerve damage has been related to endothelial damage/dysfunction. This presentation explores the role of various ocular and systemic circulatory factors in the pathogenesis of glaucomatous neuropathy.

■ 2352

Most readily usable methods to measure ocular blood flow

GUGLETA K

University Eye Hospital, Basel

Purpose SIS Lecture.

Methods Literature search.

Results Ocular Blood Flow Research Association (OBFRA, recently merged with another organization - ISOCO, into one single Association for Ocular Circulation - AOC) made a significant contribution to standardization of the blood flow measuring techniques in the field of ophthalmology. A consensus was reached on the number of OBF measurements techniques that occurred in the past decades. Particular emphasis was placed on the basic technology, specific parameters and interpretation, accuracy and reproducibility, field of clinical applications. Open questions were extensively discussed, limits of each technique clearly postulated, and a consensus statement put together for each of the technique involved. It encompassed techniques like color Doppler imaging, laser Doppler flowmetry (continuous as well as scanning LDF), laser Doppler velocimetry, Retinal Vessel Analyzer, combination of the vessel diameter measurement and the LDV, laser interferometry of the fundus pulsations amplitude, retinal oxymetry, measurements of the pulsatile component of the blood flow, blue field entoptic method and the newest - Doppler OCT.

Conclusion There is no overwhelming measuring technique able to cover all the aspects of the research and the daily clinical routine. Various parameters and various vascular beds are involved, which makes the interpretation of the obtained results strenuous. Of particular importance is the capability of OBF measuring techniques to capture one dynamic feature of ocular circulation - its ability to regulate and to response to various challenges. It is widely believed that not the constantly reduced blood flow, but rather the lack of regulation thereof, leads to prevalent ocular diseases.

■ 2354

Impact of medication on ocular blood flow

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(2) *Biomedical Engineering and Physics, Vienna*

Purpose Reduced ocular blood flow appears to play a role in the pathophysiology of glaucoma. Hence, there is considerable interest in drugs that are capable of improving ocular perfusion.

Methods A large number of clinical trials have been performed investigating the ocular hemodynamic effects of topical and systemic medications. Such trials used a variety of different methods to assess ocular blood flow parameters.

Results When administered systemically most vasodilators decrease systemic blood pressure thereby reducing ocular perfusion pressure (OPP). Only few classes of drugs have been reported to increase ocular blood flow with no or minimal effect on OPP. Among these carbonic anhydrase inhibitors and endothelin receptor antagonists show the most pronounced ocular vasodilator effects. The ocular hemodynamic effects of topical medications is generally considered small.

Conclusion When drugs are given systemically the effects on OPP have to be considered. In addition, the potentially positive effects on ocular perfusion need to be carefully weighed against the side effects. With topically administered drugs the ocular hemodynamic effects will be generally small, because the drugs reach the posterior pole of the eye in small concentrations only.

■ 2361

Recent developments of glaucoma in children

NISCHAL KK

Great Ormond St Hospital for Children, Department of Ophthalmology, London

Pediatric glaucoma can be a very challenging area of practice. There have been four areas of advances in the last 5-10 years to help improve the outcomes of treatment. Improved pharmacological agents available have been developed for adult glaucoma some of which have been safely used in children, the exception being brimonidine. A better understanding of the dynamics of aqueous flow and how to harness this have led to a safer filtration methodology with the use of antimetabolites especially Mitomycin C. Tubes or setons have been improved in terms of design with valved tubes eg the Ahmed tube, becoming more popular. Lastly, high frequency ultrasound has led to a better understanding and also better delivery of cyclodiode laser ab externo. Endophotocoagulation has also offered increased efficacy of cycloablation.

■ 2363

Management of neuro-ophthalmology cases in children

MILAZZO S

CHU Amiens, Department of Ophthalmology, Amiens

Neuro-ophthalmological conditions in children must be analyzed carefully and some emergencies must be recognized. Clinical and practical cases are illustrated. On that basis the most important aspects of the field of neuro-ophthalmology are covered succinctly but sufficiently to enable the practitioner to determine an appropriate diagnosis and course of treatment. Rare and unusual diseases are included but those that are especially critical to the patient's vision or general health and life. Some specific clinical signs and symptoms need to be carefully evaluated as papilledema, papillary abnormalities, ptosis, diplopia or ocular motility abnormalities. Each clinical case deals with a symptom that leads the patient to seek medical attention or a sign that is demonstrated on physical examination. The proper diagnosis is frequently made with the imaging but must be repeated if necessary. Specific techniques in children are described including oculomotor and electrical testing, tomography and magnetic resonance imaging. Different treatment as medical surgical and neurosurgical are discussed. The physician evaluating neuro-ophthalmic patients needs to have a broad background of medical information. So the patient's workup is properly managed and therapeutic orientations can be done.

■ 2362

Recent developments of dry eye in children

BREMOND-GIGNAC D (1, 2)

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(2) INSERM UMRS968, Vision Institute, Paris VI University, Paris

Purpose Dry eye syndrome in children is a rare disease sometimes difficult to diagnose. The research of the etiology can contribute to adjust the treatment.

Methods Two features of dry eye syndrome in children can be distinguished. Dry eye integrated in general disease with evidence of diagnosis and etiology and asymptomatic dry eye which needs a careful check up to recognize the primary etiology. Keratitis sicca usually does not lead to children complaint and simple signs as rubbing or blinking are often observed. Clinical and practical cases are described.

Results Review of literature confirms common conditions with adult dry eye but also the specificity of the pediatric dry eye. Dry eye in children is also commonly an inflammatory condition of the ocular surface. New treatment with their indications are listed including tear-like topical therapies and anti-inflammatory or immunosuppressive drugs for ocular surface with specificities in children.

Conclusion Dry eye syndrome in children must be recognized and diagnosed. In addition to classical tear-like treatment, specific therapy should be adapted to the etiology.

■ 2364

Recent developments of ocular allergy in children

CHIAMBARETTA F

CHU Clermont Ferrand, Department of Ophthalmology, Clermont Ferrand

First we summarize the clinical presentations associated with the classification of ocular allergy, and present the last agreement for treatment. Vernal keratoconjunctivitis is the more severe form affecting children and iatrogenic complications must be avoided. In vernal keratoconjunctivitis, a new grading system is introduced based on clinical signs and symptoms of ocular surface inflammation. Based on this new grading of vernal keratoconjunctivitis, different treatment options are proposed, essentially steroid sparing. Based on new information about the pathogenesis of vernal keratoconjunctivitis, recent and more selective drugs like anti-chemokine receptor antibodies and leukotriene receptor antagonists and cyclosporine are evaluated.

■ 2365

Recent developments in retinopathy of prematurity

MORTEMOUSQUIE B

CHU Bordeaux, Ophthalmology Department, Bordeaux

The retinopathy remains the principal severe ophthalmologic complication of neonates with a gestational age of 32 weeks or less. It's a major cause of lifelong blindness beginning in infancy. As many other ocular pathologies, including diabetic retinopathy, and age-related macular degeneration, result in vision loss because of aberrant neoangiogenesis. A common feature of these conditions is the presence of hypoxic areas and overexpression of the proangiogenic vascular endothelial growth factor (VEGF). Its prevention can be made by a better management of the oxygenation of these children but also by a better knowledge of the other risk factors. The prevailing current treatment, laser ablation of the retina, is destructive and only partially effective. Preventive and less destructive therapies are much more desirable. So, Angiogenesis, or the formation of new retinal blood vessels is a key feature of many proliferative retinal diseases including diabetic retinopathy, retinal vein occlusions, and retinopathy of prematurity.

■ 2371

Concordia pro visu: initiative, purpose and developments

KIVELÄ T

Helsinki University Central Hospital, Helsinki

Purpose To present the idea behind the Concordia collaboration, a joint portal for European education in ophthalmology, for those who want to promote European ophthalmology.

Methods Personal remembrances.

Results The field of European ophthalmology has been characterised by proliferation of new societies and congresses ever since its birth, perhaps more than that of its counterparts over the world. In an effort of strengthening Europe as a center for education and research in ophthalmology, the idea was born to bring together major societies that cover all ophthalmological subspecialties and the whole of Europe, especially in the field of education, so as to increase collaboration and to avoid duplicating efforts and wasting resources. To avoid creating yet another society, a virtual portal, Concordia pro visu, was designed as a common entry site into European education in ophthalmology. This page now replaces the previous home pages of European Board of Ophthalmology (EBO), European Society of Ophthalmology (SOE) and European University Professors in Ophthalmology (EUPO), the Concordia partners, and is closely linked with the EVER home page, its scientific partner. From the virtual portal, the Concordia collaboration has expanded to joint symposia and joint marketing and publicity of European ophthalmology in international and national congresses. The idea behind Concordia - working together for the benefit of vision - has proved viable indeed.

Conclusion Collaboration and convergence are necessary to paragon European ophthalmology in face of natural divergence induced e.g. by increasing subspecialisation.

■ 2372

EBO, ENET and accredited courses

TASSIGNON MJ

Antwerp

ABSTRACT NOT PROVIDED

■ 2373

EUPO courses

SPILEERS W

Dept of Ophthalmology, Leuven

EUPO (European Professors of Ophthalmology) organizes yearly courses especially for European residents in ophthalmology. The actual status and the planning for the near future of the activities of EUPO will be highlighted. The close conjunction with SOE, EBO and EVER will be outlined.

■ 2374

SOE and its role in Concordia

SEREGARDS

St Eriks Eye Hospital/Karolinska Institutet, Stockholm

Purpose To review the role of European Society of Ophthalmology (SOE) and its role in the integration of European Ophthalmology and the Concordia initiative

Methods Review of records and SOE Congress Programmes combined with interviews of key leaders.

Results The European Society of Ophthalmology currently have 42 member societies (national ophthalmic societies). The SOE is largely confined to geographical Europe but notably also includes three associate member societies of Jordan, Egypt and Israel. The SOE strongly supports the Concordia initiative. Current work includes the revision of statutes to be able to work more closely with membership societies and subspecialty societies. Also, the biennial Congress has now been transformed to a "platform" for subspecialty, educational and research societies to further integrate European ophthalmology. The SOE strongly believe that for European integration to be lasting it should be designed "bottom-up" based on common goals.

Conclusion The SOE support the Concordia initiative and further integration of European ophthalmology.

■ 2375

EVER and emphasis of research in teaching

RIVA CE
Bologna

One of the biggest challenges faced by the current medical professionals is to keep current with clinical information to practice evidence-based medicine. Conducting research during the clinical training will help future physicians better understand their field and the latest clinical developments. In this context, this presentation will emphasize what can be specifically gained by being exposed to research in a laboratory: a deeper understanding of one's chosen discipline; knowledge of on how research is conducted in one's discipline; be exposed to creative problem solving; learn how to apply concepts to real time situations, present findings at conferences; read more critically the pertinent literature and others.

■ 2376

Current methods of teaching ocular microsurgery

ZAGORSKI ZF
Lublin Medical University, Ophthalmology, Lublin

Purpose To present methods of teaching residents ocular surgery and present the future needs.

Methods Reviewing the existing methods from the literature and selected national society programmes, including the own experience.

Results The important features of surgical training programmes are:

1. Selection of trainees,
2. Training methodology,
3. Assessment of results,
4. Disclosure to patients that residents are involved in surgery and informed consent.

The training methodology comprises of knowledge of the procedure, supervised training, practical surgical exposure and practice, experience, follow-up and audit of outcomes. The knowledge comes from reading textbooks, observing videos, 3-D animation, live surgery and assisting an experienced surgeon. Supervised training should be conducted in skills centers, using practice eyes and/or surgical simulators. Assessment of surgical skills should be a part of continuous assessment of all physicians. Currently no system exists for the routine accurate assessment of surgical skills relating to surgical outcomes in clinical practice. It can be assessed objectively using surgical simulators and by analyzing the database of all surgeries performed by residents and developing an evaluating form of residents surgical skills (OASIS). Patients should be informed that residents may be involved in their surgery in order to avoid possible litigation.

Conclusion

1. Reliable methods of candidate selection and assessment need to be developed and established.
2. Continuous assessment of all surgeons and trainers should be introduced.
3. Skills centers and modern training technologies should be available to all trainees.
4. The above considerations could be included in the development of CME programs concerning the improvement of surgical skills

■ 2411

Management of macular edema due to branch retinal vein occlusion with intravitreal injections of pegaptanib sodium

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(2) University of Valencia. Faculty of Medicine, Valencia

Purpose To evaluate the efficacy of Pegaptanib Sodium as single therapy for macular edema due to non-ischemic branch retinal vein occlusion.

Methods In this uncontrolled prospective pilot study 17 patients have been included with non-ischemic branch retinal vein occlusion. All of them showed macular edema in the OCT with central foveal thickness higher than 350 microns (mean, 541 +/- 140) and decrease in visual acuity lower than 0,5 Snellen ETDRS charts notation (mean, 0,16 +/- 0,14). After an initial injection of Pegaptanib Sodium evaluations were programmed every 5 weeks and further retreatments were developed in cases with macular thickness higher than 300 microns. No other therapies were allowed at any point of the study.

Results During the follow-up a mean number of 4,3 injections were needed to achieve a mean visual acuity of 0,35 +/- 0,28 and a mean macular thickness of 264 +/- 73 microns. All patients showed an improvement in both visual acuity and macular thickness. No systemic or ocular adverse event was registered.

Conclusion Intravitreal injections of Pegaptanib Sodium constitute a safe and effective new approach in the treatment of macular edema due to non-ischemic branch retinal vein occlusion. Further controlled prospective trials are required to confirm our preliminary results.

■ 2413

VEGF is reduced in breast milk after intravitreal injection of bevacizumab

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Purpose To measure the level of VEGF protein in serum and breast milk after intravitreal injection of bevacizumab and ranibizumab.

Methods Serum and breast milk samples before and after repeated intravitreal injections of bevacizumab and ranibizumab were collected from a 35-year old female who was treated for CNV secondary to a chorioretinal scar and who was nursing her 4-month old son. Samples were analysed for the level of VEGF protein via ELISA.

Results VEGF protein in the serum was reduced significantly to non-detectable levels after intravitreal injection of bevacizumab and started to recover only after 6 weeks. After intravitreal injection of ranibizumab, however, only a temporary dip 3 days after injection could be detected. VEGF in breast milk was reduced by approximately one third directly after injection of bevacizumab. After injection of ranibizumab, the level of VEGF protein remained unaltered.

Conclusion Use of intravitreal VEGF inhibitors should be avoided during pregnancy or while nursing a baby. However, if necessary, ranibizumab seems to have a lower effect on VEGF in the serum or breast milk and should therefore be used.

■ 2412

Myopic choroidal neovascularization treated by intravitreal bevacizumab: comparison of two different initial doses

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Purpose To report the anatomical and visual outcome of myopic choroidal neovascularization (CNV) treated by two different schedules with intravitreal bevacizumab.

Methods Prospective, comparative, consecutive, non randomized, multicentric, interventional pilot study. Two groups of highly myopic patients with subfoveal and juxtafoveal CNV were treated by monthly intravitreal injections with 1.25 mg bevacizumab. Group 1 comprised 19 eyes treated by three consecutive monthly intravitreal injections. Group 2 comprised 20 eyes treated by one single intravitreal injection. Patients were evaluated for best corrected visual acuity (BCVA) and optical coherence tomography (OCT) at baseline and then monthly. Fluorescein angiography was performed at baseline and when CNV activity was suspected. Further intravitreal injections were performed if CNV activity was detected.

Results Both groups were matched for age, spherical equivalent, LogMAR BCVA, central foveal thickness (CFT) as determined by OCT at baseline and number of eyes with previous PDT treatment. The average number of letters gained was 6.3 in Group 1 vs 7.2 in Group 2 (p=0.001 and 0.09 respectively, Student t test for paired data). Changes in OCT were not significant for either group by the end of follow up. The mean number of total injections were 3.2 (Group 1) vs 1.7 (Group 2) (p=0.00, Mann-Whitney test). Four recurrences (four eyes) occurred in Group 1 vs fifteen (seven eyes) in Group 2 (p=0.001; Fisher exact test).

Conclusion Both initial treatment have similar results improving BCVA. Group 1 was treated by a higher number of injections achieving a longer lasting inactivation of myopic CNV, reducing the number of recurrences during the first year.

■ 2414

Inflammatory factors in experimental retinal vein occlusion

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Purpose Vascular endothelial growth factor (VEGF) is a key player in the development of macular edema (ME) in patients with retinal vein occlusion (RVO). In addition to VEGF, various other factors are altered in the hypoxic retina and influence the development of ME. In a rat model of RVO, we determined the changes in the gene expression of factors implicated in the development/resolution of ME (VEGF-A, PEDF, IL-6, IL-1 β , potassium and water channels) in the retina and pigment epithelium (RPE).

Methods In one eye of Long-Evans rats (n=30), retinal veins near the optic nerve head were photocoagulated using a blue-green argon laser. Untreated eyes served as controls. The eyes were enucleated 1, 3 and 7 days after laser treatment, and the mRNA levels were determined with real-time PCR.

Results In the neural retina, VEGF expression showed fast upregulation at 1 day after RVO, and returned to the control level after 3 days. The expression of VEGF was not altered in the RPE. Potassium and water channels were downregulated in both tissues through the entire time period investigated. IL-6 and IL-1 β were rapidly and strongly upregulated in the retina and RPE (to levels 80-times higher as control) within 1 day after RVO, and decreased only slowly after 7 days.

Conclusion In RVO, there is fast and transient upregulation of the expression of VEGF, which is dominant for the acute stage of the disease. The strong and long-lasting upregulation of IL-6 and IL-1 β suggests that inflammatory factors play an important role in chronic hypoxia.

■ 2415

Low dose verteporfin (1.5mg/m²) in chronic idiopathic central serous chorioretinopathy

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Purpose In chronic idiopathic central serous chorioretinopathy (ICSC), recent studies indicate that photodynamic therapy (PDT) could be effective in subretinal fluid resorption with reduced dose of verteporfin (3mg/m²) and reduced fluence PDT. However safety and cost issues remain to be addressed. This prospective randomized controlled study has been designed to evaluate the efficacy of a further reduced dose of verteporfin for PDT in chronic ICSC.

Methods Patients with chronic ICSC greater than 3 months of documented duration were included. Visual acuity had to be <20/40. The patients were randomly assigned to 2 treatment groups. They received either 3 mg/m² or 1,5 mg/m² verteporfin and reduced fluence PDT. All patients had visual acuity, ocular coherent tomography (OCT), fluorescein and indocyanine green (ICG) angiography on inclusion, follow up was based on ETDRS and OCT at 6 weeks, 3 and 6 months.

Results Ten patients were included, randomized to each treatment group. Mean duration of symptoms prior to PDT was 6 months. After 6 month follow-up, no significant difference between the 2 groups was observed: the reduction of mean foveal thickness was equivalent in both groups. In all treated eyes, visual acuity remained stable or improved. OCT showed a reduction in serous detachment in 2 eyes of 10 eyes, complete resolution was demonstrated in 8 of 10 eyes. None of the eyes required more than 1 treatment for recurrent serous detachment.

Conclusion Our preliminary data suggest that PDT leads to resolution of serous detachments and visual improvement in patients with chronic ICSC even with a low dose of verteporfin. This will reduce the cost of PDT for each patient. Long term follow-up should enable to address safety issues.

■ 2417 / 428

Intravitreal bevacizumab as primary local treatment for choroidal neovascularization secondary to uveitis

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Purpose To report short term results of intravitreal (IVT) bevacizumab as primary local treatment for choroidal neovascularization (CNV) secondary to uveitis.

Methods Files of uveitic patients receiving one or more 1.25mg/0,05ml IVT bevacizumab treatment for CNV were reviewed for clinical findings, best-corrected visual acuity (BCVA), fluorescein angiography (FA) and optical coherence tomography (OCT), concurrent treatments, number and frequency of IVT bevacizumab and treatment related adverse events. Patients previously treated with any local therapy were excluded.

Results 15 patients included. Underlying diagnosis: multifocal choroiditis and panuveitis in 7, ampiginous choroiditis in 2, and for 6 remaining, serpiginous choroiditis, sympathetic ophthalmia, Vogt-Koyanagi-Harada syndrome, punctate inner choroidopathy, tuberculous uveitis and idiopathic inflammation respectively. Subfoveal neovascularization in 13 eyes, peripapillary in 2. No active intraocular inflammation by time injections were given. 86,66% had a significant decrease in central foveal thickness by the end of follow-up (mean pre-treatment OCT 239,06µm, mean post-treatment OCT 195,2µm). BCVA improved in 80% of eyes at last follow-up (mean pre-treatment BCVA logMar 0,53; mean post-treatment BCVA logMar 0,29). 12 eyes received more than one IVT bevacizumab, mean number of injections in this group was 4,25(2-8), with a frequency of 1 injection every 13,68 weeks. No adverse effect related to bevacizumab nor to the injection procedure. Median follow-up 15,93(2-25 months).

Conclusion IVT bevacizumab was safe and effective first local treatment for inflammatory CNV, in patients under adequate control of intraocular inflammation.

■ 2416 / 427

Apoptotic activity of intravitreal bevacizumab on rabbit retina

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Purpose To evaluate the safety and the apoptotic activity of intravitreal (IV) injection of bevacizumab in rabbit eyes by histopathological analysis.

Methods Fourteen rabbits were divided into three groups, 8 rabbits in the first and 3 rabbits in each of the second and the third groups. We applied 1.25 mg/0.05 ml IV bevacizumab to the right eyes of each subject in the first and the second groups and the same volume of saline to the left eyes of each subject in the first and the third groups. The left eyes in the second group and the right eyes in the third group were left untreated and used as the control group. After 14 days, all animals were sacrificed and all eyes were enucleated for histological examination.

Results There was no evidence of ocular inflammation, cataract, or retinal toxicity in any of the sections examined by light microscopy. We observed inflammatory cell infiltration that includes plasma cells in 3 of 11 eyes which received bevacizumab, 4 of 11 eyes which received saline, and 2 of 6 eyes which were left untreated. In all groups, we observed some vacuolization in the ganglion cell layer and some distinctive dispersion and detachment of retinal layers, including rod and cone cells and the retinal pigment epithelium. After immuno-histochemical staining with Caspase 3 and p53, there was no histological evidence of toxicity to the retina and the optic nerve in any of the sections that were analyzed in all three groups.

Conclusion According to the results of this study, IV injection of bevacizumab with the dose of 1.25 mg/0.05 ml caused no histological sign of toxicity or apoptotic activity on rabbit retina.

Commercial interest

■ 2418 / 429

Photodynamic therapy for symptomatic circumscribed choroidal hemangioma

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Purpose Photodynamic therapy (PDT) is a well established treatment option for symptomatic choroidal hemangioma. We present seven patients treated with PDT at our department.

Methods Seven patients (2 female, 5 male) with a mean age of 46 Years (range 44 - 69 years) have been treated between October 2005 - March 2007. Best corrected visual acuity at first visit was minimum 0.25 and maximum 0.8 (Snellen). Indirect ophthalmoscopy showed a choroidal hemangioma with subretinal fluid. Ancillary examinations included angiography, OCT and A- and B-scan ultrasonography. Largest basal diameter was 8 mm (median; range 5.5 - 10.2 mm) and tumor height 3 mm (median; range 2.2 - 4 mm). One or more PDTs had to be done. Median follow up was 19 months (range 3 to 39 months).

Results In 3 patients one PDT was sufficient to obtain good and stable visual acuity. Three patients needed a second PDT twelve months after the initial treatment. In one patient six PDTs were necessary over a period of 20 months and additional vitrectomy had to be done. Five patients showed a gain in visual acuity (median 4 lines; range 1 to 6 lines). One patient showed unchanged visual acuity and one patient showed a loss of 3 lines after six PDTs and vitrectomy.

Conclusion In six out of seven patients stable visual acuity could be obtained. In one patient multiple PDTs and vitrectomy were necessary to achieve stable clinical results. Our findings confirm that PDT is an effective treatment modality for choroidal hemangioma.

■ 2421

Effect of nitric oxide synthase inhibition and nerve stimulation frequency on autonomous choroidal vasodilation

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Purpose To investigate the effect of non selective nitric oxide synthase inhibition and the influence of nerve stimulation frequencies on parasympathetic choroidal vasodilation.

Methods Stimulations of parasympathetic nerve fibers of the greater petrosal nerve with 8 and 20 Hz were performed simultaneously with continuous measurements of intraocular pressure (IOP), choroidal blood flow (ChorBF), orbital venous pressure (OVP) and arterial pressure at the eye level (MAP). Stimulations were performed at baseline, after non selective inhibition of all nitric oxide synthases with L-NAME and after systemic administration of atropine in an anesthetized acute rabbit model.

Results Baseline values (mean \pm sem, n=6): MAP 66.9 \pm 1.8 mmHg, IOP 17.3 \pm 0.8 mmHg, ChorBF 467 \pm 54 P.U. Facial nerve stimulation with 8 Hz under baseline conditions causes a significant increase of ChorBF 660 \pm 80 P.U. and IOP 20.7 \pm 1.6 mmHg and a non significant decrease of MAP 61.1 \pm 5.0 mmHg. The effects of 20 Hz stimulation did not differ significantly from the 8 Hz results (MAP 68.1 \pm 5.0, IOP 22.86 \pm 2.0, ChorBF 688 \pm 73). Non selective inhibition of the nitric oxide synthases with L-NAME (20 mg/kg) changed the vasodilatory effects of the two stimulation frequencies. The relative effect of 8 Hz stimulation was significantly smaller than the effect of 20 Hz stimulation. However, baseline blood flow after L-NAME was lower than under baseline conditions.

Conclusion 8Hz and 20 Hz parasympathetic stimulation had similar effects before but not after non selective NOS inhibition. However, no dose of L-NAME completely suppressed the effect of parasympathetic stimulation.

■ 2423

Effect of breathing a mixture of 92% O₂ + 8% CO₂ on flicker induced vasodilatation

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Purpose It has been shown that increased neural activity evoked by stimulation with diffuse luminance flicker increases retinal and optic nerve head blood flow. Beside others, an increased oxygen demand has been attributed to evoke the flicker response. This study seeks to investigate whether the flicker light induced increase in retinal vessel diameters is different in subjects breathing 92% O₂ + 8% CO₂ compared to breathing room air.

Methods 24 healthy volunteers were included in the study. Diameters of retinal vessels were recorded continuously with a Retinal Vessel Analyzer. During this measurement flicker stimulation was applied at a frequency of 8 Hz. Subjects were breathing a combination of 92% O₂ + 8% CO₂ and room air in a randomized, two way cross over design. Flicker responses were assessed during the two breathing periods. Blood gas values were determined from capillary blood samples.

Results Under room air conditions flicker stimulation significantly increased retinal venous diameters (p<0.05). Breathing of 92% O₂ + 8% CO₂ increased pO₂ from 88 \pm 18 mmHg to 277 \pm 71 mmHg (p<0.05) and pCO₂ from 37 \pm 3 mmHg to 46 \pm 6 mmHg (p<0.05). Breathing a combination of 92% O₂ + 8% CO₂ significantly increased flicker induced vasodilatation in retinal veins compared to room air (p<0.05).

Conclusion Breathing of a combination of 92% O₂ + 8% CO₂ increases the response of retinal venous diameters to stimulation with flicker light. The reason for this effect has, however, yet to be clarified.

■ 2422

Microstructural alterations of the retinal arterial blood column along the vessel axis in healthy volunteers with age

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Purpose We demonstrated previously that roughness of the retinal arterial blood column measured along the vessel axis increases in anamnestically healthy volunteers with increasing age. We termed it longitudinal retinal arterial profile (LAP). Whether LAP is altered with age in medically supervised healthy persons is investigated.

Methods 82 medically healthy volunteers were examined by Dynamic Vessel Analyzer (IMEDOS, Jena, Germany) using stimulation with flickering light. 3 age groups were formed: young (N=27, 30.5 \pm 4.3 years), middle age (N=28, 42.3 \pm 3.3 years) and seniors (N=27, 64.0 \pm 5.0 years). Included in the analysis were volunteers without medical vascular risk factors defined as: blood pressure < 140/90 mmHg, HDL > 35 mg/dl, LDL < 190 mg/dl and glucose levels < 110 mg/dl. Retinal arterial diameters were measured along 1 mm vessel segments to obtain LAP. Differences were analyzed using Fourier transformation.

Results In all age groups LAP do not change during all stages of the arterial response. Arterial diameters in the senior group were reduced in comparison to the young group at all stages of the vessel reaction (p<0.05). There are differences in LAP between the age groups. Compared to young persons, seniors showed significantly diminished waves with a period of 417 μ m at all stages of the arterial reaction, whereas young volunteers showed less pronounced waves with a period of 208 μ m (p<0.05).

Conclusion Our results represent the healthy aging process in retinal vasculature. Age related microstructural changes in longitudinal profiles of retinal arteries in medically healthy persons might be an indication for alterations in the vascular endothelium and smooth musculature.

■ 2424

Effects of moxaverine on ocular blood flow in patients with age-related macular degeneration, patients with primary open angle glaucoma and in healthy controls

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Purpose Several common eye diseases including age-related macular degeneration (AMD) and primary open angle glaucoma (POAG) are associated with ocular perfusion abnormalities. Moxaverine has been shown to increase ocular blood flow in young, healthy volunteers after intravenous administration. The present study investigated whether moxaverine alters ocular blood flow in elderly patients with AMD or POAG and in healthy control subjects.

Methods 20 patients with AMD, 20 patients with POAG and 20 age-matched healthy subjects were included in this trial. 150 mg moxaverine (Ursapharm, Saarbrücken, Germany) was administered intravenously over 30 minutes. Systemic haemodynamics, retinal vessel diameters, choroidal, optic nerve head and retrolubar blood flow were measured before and up to 90 minutes after drug administration.

Results Administration of moxaverine increased choroidal blood flow by 8.7 \pm 21.8% (p=0.012) and optic nerve head blood flow by 12.9 \pm 33.3% (p=0.021). Additionally, an increase in the mean flow velocities of posterior ciliary arteries (24.8 \pm 34.7%, p<0.001) and in the ophthalmic artery (23.3 \pm 33.5%, p<0.001) was found after administration of moxaverine. However, no differences were found between the 3 study groups. No significant change of retinal vessel diameters was observed.

Conclusion The present study indicates an increase of ocular blood flow after systemic administration of a single dose of moxaverine in patients with POAG, patients with AMD and in age-matched healthy controls. Further studies are needed to investigate possible beneficial effects after long-term treatment in patients with ocular diseases associated with hypoperfusion.

■ 2425

Effect of glaucoma and glaucoma risk factors on choroidal hemodynamics

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Purpose a) to determine subfoveal choroidal hemodynamics in patients with primary open angle glaucoma (POAG) and patients with ocular hypertension (OH); b) to assess the effects of diabetes (DM), systemic hypertension (SHT) and myopia on subfoveal choroidal hemodynamics

Methods Laser Doppler flowmetry (LDF) was used to determine the subfoveal choroidal blood velocity (ChBVel), volume (ChBVol), and flow (ChBF) in 1) patients with POAG (n=85) and patients with OHT (n=25); 2) patients with glaucoma risk factors which were further subdivided into three subgroups; DM (n=93), SHT (n=57) and myopia (n=29) respectively. Subjects with each risk factor were further subdivided into two subgroups (without and with POAG), 3) age matched healthy controls (n=100).

Results All LDF parameters were significantly reduced in all groups of patients compared with age matched controls. No statistically significant differences in the LDF parameters among HTG, NTG and OHT subgroups were detected. No significant difference in the LDF parameters between the two subgroup of each risk factor (without and with POAG) was noted. The LDF data of glaucomatous patients with risk factors demonstrated a significant reduction of ChBF and an increase in resistance in comparison to glaucomatous patients without risk factors

Conclusion Subfoveal choroidal LDF parameters are reduced in subjects with POAG, OHT and patients with glaucoma risk factors, such as DM, SHT (under antihypertensive therapy) and myopia when compared with age matched healthy controls. However, the role of these choroidal circulatory alterations in the development or progression of the glaucomatous optic neuropathy remains to be clarified.

■ 2427 / 260

Consequences of dietary omega-3 polyunsaturated fatty acid deficiency on retinal function and intraocular pressure in the rat

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Purpose Omega-3 polyunsaturated fatty acids (ω 3) are key components in nervous structures but their dietary intakes in the overall population are often below nutritional requirements. A chronic deficiency in ω 3 is recognized to be associated with functional impairment of the retina. At the opposite, ω 3 supplementation is associated with a reduced risk for AMD. The consequences of ω 3 deficiency on other eye structures than the retina, such as ciliary bodies, are scarce. The purpose of our study was to compare the response of the retina and ciliary bodies to dietary ω 3 deficiency in terms of fatty acid profile and eye functionality.

Methods Two successive generations of Lewis rats (G1 and G2) were obtained under either a standard or ω 3-deficient diet. Intraocular pressure (IOP) was measured by rebound tonometry throughout the experiment. Retinal functionality was assessed by scotopic electroretinography (ERG). Gas chromatography was used to determine the fatty acid profile of the ciliary bodies and retina.

Results A 2-fold fall in DHA content of the retina was observed in ω 3-deficient G1 animals. This decrease was accentuated in G2 (-66%) and counterbalanced by an increase in DPA ω 6 in the retina. The b-wave amplitude of the ERG was decreased by 50% at 9mcds/m² in ω 3-deficient rats. In ciliary body DHA was reduced by 80% in ω 3-deficient in G1 animals but not in G2. Meanwhile, animals from the ω 3-deficient diet had increased IOP (18 vs 12mmHg, p<0.0001).

Conclusion The crucial role of ω 3 in retinal function was confirmed. The most relevant finding from our study is the rise in IOP, the major risk factor for glaucoma, which was observed in animals reared under dietary deficiency in ω 3.

■ 2426 / 259

In vitro transcorneal and transscleral diffusion of radiolabeled compounds in human and rabbit cornea and in human, monkey, dog, and rabbit sclera

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Purpose To determine the in vitro diffusion of selected compounds across the cornea or sclera of humans, monkeys, dogs, and rabbits.

Methods Human and NZW rabbit corneas were obtained and the epithelium was removed from one cornea of each pair. Corneas were mounted in chambers with 3H-water or 3H-dexamethasone on the epithelial side and BSS solution on the endothelial side. Serial aliquots were taken from each chamber and assayed by LSC. Scleral sections from human, NZW rabbit, dog, or monkey eyes were mounted in perfusion chambers. Adjacent sclera was used for H&E histology. 3H-water, 3H-dexamethasone, or 70kD-14C-dextran were added to the episcleral surface while perfusing BSS across the choroidal side. Serial aliquots were collected up to 5 hrs and assayed by LSC. Scleral permeability (ktrans) was calculated. H&E slides were used to determine scleral thickness.

Results 3H-water diffused through cornea faster than 3H-dexamethasone, especially with denuded epithelium. Scleral thickness and molecular weight were determinant of diffusion in sclera. Monkey sclera was thinnest, followed by dog, rabbit, and human. ktrans for 70kD-14C-Dextran and 3H-dexamethasone were greatest in monkey, followed by dog, rabbit, and human. ktrans values for 3H-water were similar in all species, and greater than values for 14C-dextran and 3H-dexamethasone.

Conclusion These studies demonstrate permeability of cornea and sclera in human and animal models with compounds of varied molecular weights representative of drugs being developed for treatment of ocular diseases. The results of this study indicate that these techniques are valuable as screening tools in the development of ocular drugs.

■ 2431

Visual function after cataract surgery in patients with an aspherical lens without spherical aberration

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Purpose To compare quality of vision in pseudophakic patients with customized-asphericity intraocular lens (IOL) compared to patients with zero-aberration IOL after a Micro-Incision Cataract Surgery (MICS).

Methods 26 patients (41 eyes) were divided into two groups: 24 eyes received a customized-aspheric IOL in both eyes and 17 eyes received zero-aberration IOLs. IOL asphericity was customized according to the preoperative corneal spherical aberration (SA) in order to obtain a total ocular SA close to +0 μm . We implanted either aspherical AcriSmart 36A⁺ generating a -0,18 μm SA (for a 6 mm pupil diameter), or zero-aberration AcriSmart 46LC⁺. Postoperative evaluations were conducted 6 months after MICS: refraction, best-corrected visual acuity (BCVA), contrast sensitivities, point spread function (PSF), Modulation Transfer Function (MTF), objective depth-of-focus and ocular wavefront aberrations were analyzed.

Results Postoperative BCVA was similar in both groups (-0,021 \pm 0,105 LogMAR for the custom group versus 0 \pm 0,11 LogMAR, p=0,58). Mesopic contrast sensitivity was significantly better in the custom group at intermediate and high spatial frequencies (p<0.001). SA was significantly lower in the custom group (Z40 = 0,085 \pm 0,07 μm vs 0,261 \pm 0,09 μm , p<0.001) whereas no difference of preoperative corneal SA was noted. MTF cutoff frequency is lower in the reference group than in the custom group (p=0,008). The objective residual accommodative amplitude was lower in the custom group: 1,31 \pm 0,54 D versus 2,25 \pm 0,65 D (p=0,006).

Conclusion Individual selection of IOL asphericity with a preoperative corneal spherical aberration measurement allowed control of final total amount of spherical aberration. Such a customization improved mesopic contrast sensitivity, and leads to better objective quality of vision.

■ 2433

Wavefront aberrations variations with accommodation

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Purpose To study the effects of phenylephrine 5% topical administration on accommodative response and wavefront aberrations variation.

Methods The research followed the tenets of the Declaration of Helsinki . 28 eyes from 14 volunteers with spherical equivalent defocus error between -2D and +1D, no eye disease history and between 20 to 25 years of age, underwent wavefront measurements with a Shack-Hartmann wavefront aberrometer which included a movable accommodative target. Wavefront data was acquired while applying 6 different increasing accommodative stimuli, from 0 D to 5 D by steps of 1 D, before and after pupil dilation with Phenylephrine 5%.

Results The dilation using Phenylephrine 5% was found to larger lag errors in the accommodative response of about half of the subjects. The total RMS amount of aberrations above defocus remained stable during accommodation with and without dilation. Spherical aberration was positive in average in the non accommodated eye and changed toward negative values with increasing accommodation (p<0.05). Cylinder axis came closer to 90° as accommodation increased (p<0.05). Although vertical coma did not significantly vary with accommodation, horizontal coma increased significantly with accommodation (p<0.05). These changes in aberrations with increasing accommodation were similar on average in both pupil conditions

Conclusion Phenylephrine 5% modifies the accommodative focus response of a significant proportion of young adult eyes. Wavefront aberrations above defocus undergo similar variations in both natural and dilated pupil conditions. However the total RMS error of aberrations above defocus remains relatively constant when the eye accommodates.

■ 2432

Wavefront aberrometry advances in refractive surgery

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Wavefront aberrometry has been adapted from optical astronomy in order to analyze complex image distortions caused by the human optical system, called higher order aberrations. The aberrations increase with larger pupil size or during nocturnal vision when the pupil dilates. They may have a significant impact on vision, even in a patient with 20/20 visual acuity. Most Shack Hartman aberrometers can be used as a super auto refracto-keratometer during refractive surgery pre-operative evaluations. They can capture the full amount of astigmatism and so will minimize the need for retreatment due to undercorrection. Modern Shack Hartman aberrometers give also the refractive surgeons the possibility to measure aberrations induced by flap creations. In addition they will be useful for performing customized excimer laser ablation (data coming from the aberrometer mapping out a path for the laser in reshaping the cornea). This technique has been shown to improve the correction of lower order aberrations (90% of the aberrations of the human eye), but is there a real benefit for treating higher order aberrations? Is a Shack Hartman aberrometer needed for every day refractive surgery practice? Thru an evidence based review of the literature confronted to personal experience we will try to answer these questions.

■ 2434

Adaptive optics applied to the vision simulator

CHATEAU N

Orsay

ABSTRACT NOT PROVIDED

■ 2435

Custom wavefront contact lenses

LOPEZ-GIL N
Murcia

ABSTRACT NOT PROVIDED

■ 2436

Colorimetric comparison between different filtered IOLs and human crystalline lenses at various ages

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Purpose To perform the colorimetric characterization of two different blue light filtered IOLs (Intra Ocular Lenses, from Alcon, USA and Hoya Corporation, Japan) and one UV-violet filtered IOL (Bausch & Lomb, USA) comparing them with standard UV filtered IOL (Alcon) and human crystalline lenses at various ages.

Methods Three IOLs of each model with different dioptric power (16, 21 and 26 D) were analyzed. The colorimetric coordinates of the IOLs were calculated by measuring their transmission spectra by a spectrophotometric technique. Consequently, we defined a parameter (ΔE) proportional to the perceived color differences. A comparison was then performed between the various IOLs and human crystalline lenses of different ages (4, 22, 41, 53 and 72 years) considered from the literature. The CIE (Commission Internationale de l'Éclairage) standards were adopted for the colorimetric analysis.

Results The color distance ΔE was calculated for the 4 IOL models considered, respect to human lenses at different ages between 4 and 72 years. The maximum ΔE value was found respect to the standard UV filtered IOL for any other crystalline lens age and greatest for a human 72 year old lens. The minimum value for ΔE was obtained for both blue-filtered IOLs respect to middle-aged human lenses.

Conclusion None of the analyzed IOLs has the same colorimetric performance of the human crystalline lens. The two blue-filtered IOLs reproduce at best the human lens yellowing of 20 to 50 years, among all the tested IOLs.

■ 2441

The effect of upregulated LOX and LOXL2 on inflammation and fibrosis in a laser induced CNV model

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Purpose Lysyl oxidase (LOX) and lysyl oxidase-like protein 2 (LOXL2) are involved in the cross-linking of collagen and elastin in the extracellular space. Therefore, these proteins play a major role in the process of fibrosis. This study was designed to elucidate the role of LOX and LOXL2 in inflammation and fibrosis after choroidal neovascularization (CNV).

Methods CNV was induced in 8 to 10 weeks old C75Bl/6 mice (n=5 per timepoint), by placing 3 laser spots at 9, 12 and 3 o'clock position (50µm, 0.1s and 400mW). Mice were sacrificed 2, 4, 7, 14, 28 and 35 days after laser. LOX and LOXL2 expression in choroid and retina was analyzed using real time RT-PCR. Inflammation and fibrosis was studied by different (immuno)stainings.

Results Both LOX and LOXL2 were significantly increased over time in the choroid and retina of lasered mice. LOX was 2.1, 3.2, 1.75, 1.3, 2 and 1.3 times upregulated on days 2, 4, 7, 14, 28 and 35 after laser, respectively, compared to non-lasered eyes. LOXL2 was 1.1, 1.6, 1.3, 1.1, 1.5 and 1.2 times upregulated on day 2, 4, 7, 14, 28 and 35 after laser, respectively, versus non-lasered eyes. On day 2 and 4 after the laser, the number of inflammatory cells was increased with 16% compared to control. A significant increase of collagen deposition was shown in the choroid on days 14, 28 and 35.

Conclusion A biphasic upregulation of LOX and LOXL2 was observed after CNV induction, which was associated with the inflammatory and fibrotic phase. Our data suggest that LOX and LOXL2 play a role in the process of inflammation and fibrosis after the induction of CNV. This finding can open new perspectives in the treatment of age-related macular degeneration by inhibiting LOX and LOXL.

■ 2443

Intraocular pressure and associated factors in a Central Indian population. The Central India Eye and Medical Study

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Purpose To evaluate the intraocular pressure (IOP) and its associated factors in the adult population of rural India.

Methods The Central India Eye and Medical Study is a population-based study performed in a rural region close to Nagpur in Central India. It included 4711 subjects (aged 30+ years) out of 5885 eligible subjects (response rate: 80.1%). The participants underwent a detailed ophthalmic and medical examination. This study was focused on the IOP.

Results Out of the 4711 subjects (9422 eyes), IOP measurements were available for 9338 (99.1%) eyes of 4686 (99.5%) subjects. The mean IOP was 13.6±3.4 mm Hg (median: 14 mm Hg; range: 2–56 mm Hg). Assuming a Gaussian distribution curve, the normal range of IOP, defined as mean ± two standard deviations, was from 6.8 mm Hg to 20.4 mm Hg. In multivariate analysis, IOP was significantly associated with the systemic parameters of higher diastolic blood pressure (P<0.001), higher pulse rate (P=0.004), and higher body mass index (P=0.007); the socioeconomic parameters of higher level of education (P=0.004), higher cast (P=0.002), and no livestock ownership (P=0.01); and the ocular parameters of higher corneal refractive power (P<0.001), lower central corneal thickness (P=0.002) and higher myopic refractive error (P=0.002).

Conclusion The normal range of IOP was from 7 mm Hg to 20 mm Hg. Determinants of IOP were higher diastolic blood pressure, higher pulse rate, higher body mass index, higher level of education, higher cast, higher corneal refractive power, lower central corneal thickness and higher myopic refractive error.

■ 2442

Is inhibition of VEGF165 sufficient to inhibit scar formation after trabeculectomy?

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Purpose We have previously shown that VEGF plays a role in scar formation after glaucoma surgery and that inhibition of all VEGF-isoforms by bevacizumab is able to reduce scar formation. This study was designed to elucidate the exact role of VEGF165 in scar formation after trabeculectomy. The effect of pegaptanib (Pfizer), which specifically blocks VEGF165, was investigated in vitro and in vivo.

Methods The effect of pegaptanib on Tenon fibroblasts and HUVEC in vitro was determined using a WST-1 proliferation assay. The effect of the aptamer was also investigated in vivo in a rabbit model for glaucoma surgery by studying angiogenesis, inflammation and collagen deposition.

Results A dose-dependent reduction of HUVEC proliferation was seen after pegaptanib administration in vitro (P<0.05 with a dose of at least 0.3 mg/ml). A concentration of 2 mg/ml pegaptanib was necessary to inhibit the proliferation of Tenon fibroblasts. The aptamer also significantly reduced blood vessel density 3 days after surgery in a rabbit model of trabeculectomy (P=0.001). There were no significant differences in inflammation and collagen deposition in the treated eyes compared to control.

Conclusion Whereas HUVEC cells were inhibited by pegaptanib in a dose-dependent way, Tenon fibroblasts were only inhibited at the highest dose. A single administration of pegaptanib at the time of trabeculectomy reduced postoperative angiogenesis, but not inflammation or collagen deposition. Further studies using repeated pegaptanib injections are necessary to investigate whether the lack of effect of pegaptanib on scarring was due to a shorter working time of pegaptanib compared to bevacizumab, or due to the difference in their effect on the various VEGF isoforms.

■ 2444

Screening history in those requiring fast track referral for proliferative diabetic retinopathy (PDR) in the ni diabetic retinopathy screening programme

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Purpose If PDR is detected at screening, an urgent referral to an ophthalmologist ensues. This outcome can be stressful for patients and for the ophthalmologists who may need to treat very quickly. Aim: To examine the screening history of those deemed to require an urgent referral, with a view to identifying missed cases of referable diabetic retinopathy in previous screening encounters; and risk factors for interval cases.

Methods Fast tracked urgent referrals were identified from the NI DRSP database. Demographic factors and previous screening history were analysed.

Results In 2006-7 18,887 attended for screening. 5.6% required referral to an eye clinic of which 0.3% were deemed urgent referrals (52 cases) 47 showed PDR; 5 had advanced NPDR in an only eye. 47% had Type 1 Diabetes; 52% had been diabetic for 20 years or more; 8% had been diabetic for 5 years or less. On feedback 98% were found to be appropriate referrals. At time of screening, 6 people had been lost to follow up from previous eye clinics. PDR was identified at the first screening event in the other 46 (88%). No cases were found where referral at a previous screening encounter would have been appropriate.

Conclusion Especially during the early years, screening programmes are very likely to encounter sight threatening retinopathy not previously identified. Over time, the balance between referral early in the disease process, and late, will hopefully be achieved but until then clinics must be prepared to manage the unpredictable urgent referral.

Commercial interest

■ 2445

Frequency of pterygium in indigenous Kadiweu

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Purpose Study carried out in Amazonas, north of Brazil, reported that river indigenous people have much higher rates of pterygium than indigenous living in forest. Exposure to the sun was the major risk factor for pterygium. The objective of this study was to verify the frequency of pterygium in indigenous with different ethnic and cultural background, and living in another region of the country, with different geographic characteristics (Kadiwéu indigenous).

Methods Ocular examination was performed in 10% of the total population of Kadiwéu indigenous (90 subjects), living in two small villages, in "Serra Boquena" region, State of Mato Grosso do Sul, in center-west Brazil.

Results The frequency of pterygium in all participants was 15%. Considering only individuals over 20 years the rate was 20%.

Conclusion The frequency of pterygium in indigenous Kadiwéu is lower than the rate in river indigenous and higher than in forest people.

■ 2447 / 245

Major eye diseases and risk factors associated with systemic hypertension in an adult chinese population: the Beijing Eye Study

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Purpose To assess the relationship of hypertension with major eye diseases and other ocular parameters.

Methods The Beijing Eye Study is a population-based study. Examination at baseline in 2001; follow-up examination in 2006; 3222 subjects had blood pressure measurements. All participants underwent a thorough ophthalmic examination and blood pressure measurement. Hypertension was defined as a systolic blood pressure ≥ 140 mm Hg and/or a diastolic blood pressure ≥ 90 mm Hg, and/or self-reported current treatment for hypertension with antihypertensive medication.

Results Mean age of participants in the present study was 60.4 ± 10.0 years. Hypertension was present in 1500 (46.6%) of the 3222 subjects who had their blood pressure measured. In multiple regression analysis, hypertension was associated with higher intraocular pressure ($P = 0.005$), arterio-venous nicking ($P = 0.009$), retinal vein occlusions ($P = 0.02$), and diabetic retinopathy ($P = 0.02$). Hypertension was not significantly associated with the prevalence of open-angle glaucoma ($P = 0.19$) or angle-closure glaucoma ($P = 0.15$), age-related macular degeneration ($P = 0.73$), nuclear cataract ($P = 0.88$), posterior subcapsular cataract ($P = 0.30$), cortical cataract ($P = 0.10$), or area of alpha zone ($P = 0.05$) or beta zone of parapapillary atrophy ($P = 0.95$).

Conclusion In Chinese persons, while controlling for other systemic parameters, hypertension was associated with increased intraocular pressure, retinal microvascular abnormalities, and prevalence of retinal vein occlusion and diabetic retinopathy. Hypertension was not associated significantly with age-related macular degeneration, age-related cataract, or glaucoma

■ 2446 / 244

Relationship between IOP and age in the Salzburg-Moorfields-Collaborative Glaucoma Study

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Purpose To investigate the distribution of intraocular pressure in a population based survey in the county of Salzburg.

Methods The SMC GS is a single-center, prospective cohort study embedded into a government supported glaucoma blindness prevention program in Salzburg county, Austria. A complete ophthalmological examination including biomicroscopy of the optic nerve head, visual field testing, measurements of intraocular pressure, central corneal thickness and laser scanning topography of the optic disc was performed on 3650 subjects at the beginning of the study as well as at the five year follow-up.

Results The average age is 60.3 years (min: 40.04, max: 93.9 years), IOP is 15.0 mmHg (min: 7, max: 32 mmHg) and the correlation coefficient between age and intraocular pressure is $r = 0.005$, $p = 0.75$ (95% CI -0.03 and 0.026). Also in the subgroups (people with normal findings, people with increased cup to disk ratio (C/D > 0.45) and people with ocular hypertension (IOP > 21 mmHg) no significant correlation between age and intraocular was found.

Conclusion In the past, numerous studies showed a correlation between age and intraocular pressure or at least reported a vague relationship between the two variables. Of all large scale studies only the blue mountain eye study and the Beijing eye study did not find a positive correlation between age and intraocular pressure (before correction for arterial pressure). In the population of Salzburg, age is an independent risk factor for glaucoma, independent of IOP and without correction of possible confounders.

■ 2451

Aging and glaucoma

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Purpose The rigidity of the eye increases with age. Sclera becomes stiffer and the compliance of lamina cribrosa (LC) is reduced. This is caused by modification (crosslinking) of collagens by advanced glycation endproducts (AGEs) or oxygen radicals due to oxidative stress and reduced antioxidants. Albon (2000) proved an increase of AGEs in LC with age.

Methods We showed the stiffening effect of AGEs on LC and peripapillary sclera in stress-strain measurements. Additionally, these crosslinks cause an increase of collagen resistance against degradative enzymes which leads to an accumulation of collagen in the optic nerve head. Similar changes are observed also in glaucoma, but to a higher extent in comparison to age-matched persons. The stiffness of sclera in glaucoma is higher (lower pressure buffer capacity).

Results Tezel (2006) found an increased AGE-content of 36% in the optic nerve head of glaucoma patients compared with 8% in age-matched controls. Investigations show, that these biomechanical changes are clearly demonstrated after menopause. Therefore we concluded a hormonal prevention of these age-related changes. These hormonal prevention is in glaucoma patients lower or not age-related. Studies show an elevated concentration of follicle-stimulating hormone (FSH), luteotropic hormone (LH) and a by 50% reduced estradiol amount in glaucoma patients. Estrogen does not only possess an antioxidative effect like vitamin C or E. It influences the synthesis of collagen, proteoglycans and MMPs. An adequate synthesis of glycosaminoglycans makes the tissue more flexible. Estrogen stimulates also MMPs and reduces the stiffness of tissue.

Conclusion In glaucoma the oxidative and hormone status is not age-related, which might be the cause of earlier vascular and biomechanical changes.

■ 2453 / 222

Vascular endothelial dysfunction in primary open angle glaucoma

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Purpose Glaucomatous optic neuropathy (GON) is related not only to elevated intraocular pressure (IOP) but also to an insufficient ocular blood supply related to a vascular dysregulation. We evaluated peripheral vascular endothelial function in primary open-angle glaucoma (POAG) with a non-invasive endothelium flow mediated vasodilatation (FMD).

Methods We studied 20 POAG patients. The diagnostic criteria for POAG was an IOP exceeded 22 mm/hg before treatment, open anterior chamber angle, cup/disc ratio >0.7 and optic nerve related visual field loss (MD>6 dB, SF>2.5 dB and CPSD>3 dB). Patients with systemic diseases such hypertension, heart failure and diabetes mellitus were excluded. All patients and 20 normal controls underwent measurement of FMD with high-resolution 2-dimensional ultrasonographic imaging of the brachial artery by a Philips ENVISOR echographic machine. To induce iperemia a sphygmomanometer cuff was inflated to 250 mm/hg for 5 minutes. The cuff then was deflated rapidly and at 60 seconds after cuff deflation 2-D images of the brachial artery were recorded for 5 seconds. A statistical analysis was made of the FMD in POAG and in normal control patients using the Student "t" test for unpaired data and the Pearson correlation test to show the correlation between the endothelial dysfunction and the visual field indexes.

Results Patients affected by POAG exhibit lower values of FMD compared with healthy people. In POAG patients we found an FMD 4.28 ± 2.49 vs. 8.66 ± 3.02 ($p < 0.001$). The mean decrease (MD) visual field index was related to FMD ($p < 0.05$).

Conclusion Patients with POAG have an impaired vascular function related to a vascular endothelium dysfunction and the MD visual field is related to FMD impairment.

■ 2452

Association between optic nerve head blood flow as assessed with Laser Doppler Flowmetry and mean arterial blood pressure in glaucoma, ocular hypertension and healthy control subjects

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Purpose It has been implicated that vascular dysregulation plays a role in the pathogenesis of primary open angle glaucoma (POAG). In the present study the association between optic nerve head blood flow as measured with laser Doppler flowmetry and ocular perfusion pressure in patients with treated and untreated POAG, patients with ocular hypertension and healthy control subjects was compared.

Methods 136 patients with treated POAG, 116 patients with untreated POAG, 138 patients with ocular hypertension and 160 control subjects were included in the study. Optic nerve head blood flow was assessed using laser Doppler flowmetry. Ocular perfusion pressure was calculated based on measurement of IOP and systemic hemodynamics.

Results Optic nerve head blood flow was significantly reduced in patients with glaucoma compared to patients with ocular hypertension and healthy subjects ($p < 0.01$). However, no difference in optic nerve head blood flow between treated and untreated glaucoma patients was detected. The highest association between ocular perfusion pressure and optic nerve head blood flow was found in untreated glaucoma patients followed by ocular hypertensives and treated glaucoma patients.

Conclusion The present study confirms evidence that optic nerve head blood flow is reduced in patients with POAG and patients with ocular hypertension. Correlation coefficients in the glaucoma groups and in the ocular hypertensives indicate a vascular dysregulation in these patients compared to healthy control subjects.

■ 2454 / 223

Sympathetic influence on intraocular pressure

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Purpose To apply wavelet analysis to investigate the sympathetic modulation upon intraocular pressure (IOP) of the cold pressure test

Methods In an autonomic dedicated lab continuous and simultaneous recording of (IOP) with Pascal (DCT) and arterial blood pressure (BP) were made in 11 healthy individuals, of both sexes, in basal conditions and during 1 minute of cold-pressure test (CPT). The tests were in the morning period and in the 24h previous individuals were told not to smoke and to avoid xanthenes or other stimulants ingestion. Individuals under medication or with other pathology that could affect the autonomic nervous system were excluded. Data analysis of IOP and systolic BP (SBP) in the time-scale domain with wavelets (DB12) was performed, for both variables, during the last minute prior to test and during one minute upon test. In this last case, periods of 10s where individually analysed and the period with the largest value was the chosen for statistic analysis using t-Student test, Welch corrected (Ducla-Soares et al, 2007). LFIOP and LFSBP were calculated and differences between basal and CPT values were considered significant where $p < 0.05$. Data were expressed as mean \pm SEM, and for LF mmHg²

Results Data refer to 6 male and 5 female with a mean age of 49 [40-70]. On CPT, a sympathetic challenging manoeuvre, an increase of SBP was observed. Wavelet analysis of both signals showed a significant increase of LFIOP and LFSBP from 0.13 ± 0.15 and 8.66 ± 1.51 to 0.37 ± 0.26 mmHg² and 18.7 ± 4.5 mmHg², respectively, and a not significant change in IOP 17.0 ± 2.6 to 18.0 ± 1.5

Conclusion Our data show a sympathetic signature on IOP correlated with that observed in BP. We show that wavelets are a suitable tool for the evaluation of sympathetic signature on short periods of IOP recorded by DCT

■ 2455 / 224

Sympathetic variability of intraocular pressure of glaucoma patients evaluated by wavelets

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Purpose We had showed the usefulness of wavelets in the detection of sympathetic activity in intraocular pressure (IOP). With we intend to evaluate sympathetic activity in glaucoma patients with and without topical therapeutics through the same methodology

Methods 3 groups of individuals of both sexes: 1) a healthy individuals (HI, n=11); 2) two groups of glaucoma patients with Primary Open-Angle Glaucoma, one under treatment (GT, n=25) and another without (GNT, n=10). In a autonomic Lab. Continuous recording of IOP with Pascal-DCT, in day before, individuals were told not to smoke and to avoid xanthenes or other stimulants ingestion. Glaucoma patients were diagnosed by visual fields defects and optic disc analysis. For GT patients a wash-out time of 1-3 weeks was required depending on therapeutics. Individuals under medication or any other pathology that could affect the autonomic nervous system were excluded. Data analysis of systolic IOP in the time-scale domain with wavelets (Db12) was performed during one minute. Statistical analysis using t-Student, Welch corrected (Ducla-Soares et al. 2007) was used. LF IOP was calculated for the 3 groups and difference. were consid. significant where $p < 0.05$. Data (mean \pm SEM)

Results Mean age 49 (40-70), 64 (45-67) and 61 (36-61) for HI, GT and GNT groups, respectively. The quantization of sympathetic variability of SIOP by wavelets was 0.4 ± 0.17 , 0.122 ± 0.075 and 0.118 ± 0.067 for HI, GT and GNT groups respectively. Values were significantly different between HI and GT ($p < 0.05$) and GNT ($p < 0.05$) but not between the two groups of glaucoma patients

Conclusion Our data show that glaucoma patients have a significant decrease in sympathetic activity that could be due to an extraocular mechanism that persists with therapeutics

■ 2457 / 226

Factors affecting ocular rigidity in normal human eyes

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Purpose To measure the ocular rigidity coefficient and evaluate its relation with axial length (AL), age and mean systemic blood pressure (SBP).

Methods Sixty three patients (63 eyes) undergoing cataract surgery, with different refractive errors and no ocular or systemic pathology were enrolled in this study. An invasive, computer controlled device comprising a microdosimetric pump and a pressure sensor, is connected to the anterior chamber under topical anaesthesia with drops. The system is used to raise the intraocular pressure (IOP) from 15 to 40 mmHg, by infusing the eye with a saline solution. After each 4 μ l infusion step, the IOP is continuously recorded for 2 seconds. From an initial level of 40 mmHg an IOP decay curve of 1 minute is obtained. SBP and pulse rate are measured during the procedure. The rigidity coefficient is calculated by an exponential fit to the pressure volume data after correction for outflow. The study was approved by the Institutional Board and performed under the patient's informed consent.

Results Mean AL was 24.8 (range 21.2-32.5). Mean age and SBP was 59 (12) years and 93.7 (10.5) mmHg respectively. The mean ocular rigidity coefficient was 0.021 (0.005) ul⁻¹. Increasing axial length is associated with a decrease in the rigidity coefficient ($r = -0.61$, $p < 0.01$). A positive correlation between the rigidity coefficient and age of the patients is found ($r = 0.31$, $p = 0.01$), whereas similar findings were not observed for SBP ($p > 0.05$).

Conclusion This manometric approach of measuring ocular rigidity provides a normative database of this parameter in living human eyes. Axial length and age influence ocular rigidity. These results may have implications on tonography and ocular pulse studies.

■ 2456 / 225

Measurement of IOP with radiowave telemetry

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Purpose To illustrate the implantation technique & tolerance of a novel wireless silicone-encased intraocular pressure transducer in rabbit eyes. The device can conceivably provide a reliable way to measure the IOP in situations where measurement is difficult with the present technology, for instance in eyes with keratoprosthesis implants.

Methods The transducer, manufactured by Mesotec GmbH (Hannover, Germany), is a fully digital ultra miniature system, integrating pressure sensing, data handling and telemetry on a single microchip. The microchip is connected to a telemetry coil and requires no internal power source. The data is received using an external reader unit. The transducers were initially tested for calibration in enucleated bovine eyes. They were sterilized with ethylene oxide. They were implanted either through a corneal autograft (n=2) or through a large limbal incision (n=2) and placed either in the sulcus (n=2) or suspended into the vitreous cavity (n=2) after removing the crystalline lens. In one rabbit (SHAM), the crystalline lens was removed but no implant was placed. The eyes were closed with interrupted 10-0 nylon sutures. Daily observations and weekly full clinical examinations were performed. The readings obtained by the transducer-readers were compared with those obtained by Tonopen.

Results The transducers were well tolerated, with minimal transient post-operative intraocular inflammation that was similar in both the experimental and SHAM groups at comparable time-points. The pressure obtained by the transducers showed good correlation with those obtained by Tonopen.

Conclusion Our animal studies show that the transducer can be easily implanted and is well tolerated inside the rabbit eye, up to 4 months at the present time.

■ 2458

Sensitivity of applanation tonometry readings to the geometrical parameters of the sclera and the shape of the cornea

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Purpose To study the effect of the geometrical parameters of a cornea (the thickness and difference of corneal shape from spherical segment) on applanation tonometry readings.

Methods The mathematical model for Goldmann and Maklakov tonometers is developed. The corneoscleral shell is modeled as two conjugated transversal isotropic elliptic (with non-zero eccentricity) shells (cornea and sclera) with different mechanical properties. The baseline (prior to loading) two-segment shell is assumed to be filled with incompressible liquid under pressure. Simulated IOP measurements are obtained for Goldman and 5-g and 10-g Maklakov tonometers. To analyze the effect of the eyeball geometry on IOP readings the elastic constants of cornea and sclera are varied and geometrical parameters (such as central corneal thickness, cornea and sclera radii of curvature, scleral thickness, ratio of anterior-posterior eye axis lengths vs. the equator diameter) are varied. Simulated calculations are compared with experimental data.

Results The results are obtained over a wide range of parameters of the sclera and cornea.

Conclusion If the shape of a cornea differs from spherical it could cause the measurement error of tonometry IOP readings ranged between 3% and 25%. Significant sensitivity of the Goldmann tonometer to corneal thickness compared to Maklakov's method may be explained with smaller contact zone and, hence, the larger influence of the flexural (bending) deformation, which depends on the thickness of a shell. Contrariwise the change of the radius of curvature of cornea affects greatly on the results obtained with Maklakov tonometer.

■ 2461

Retinal oxygenation in diabetic retinopathy

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Purpose Diabetic retinopathy (DR) is believed to cause retinal tissue hypoxia by damaging retinal capillaries. The purpose of this study was to examine the effect of diabetic retinopathy on oxygen saturation in retinal arterioles and venules.

Methods The retinal oximeter (Oxymap ehf., Reykjavik, Iceland) is composed of a fundus camera, beam splitter and light filters. Specialized software calculates relative oxygen saturation from light absorption at two wavelengths of light (605nm and 586nm). One first or second degree temporal arteriole and venule were measured in one eye of 31 healthy individual and 28 patients with diabetic retinopathy. The diabetic patients had background DR (n=6), macular oedema (n=7), untreated preproliferative or proliferative DR (n=7) or stable proliferative DR after treatment (n=8). Statistical analyses were performed with an unpaired t-test, one-way ANOVA and Dunnett's post test.

Results Retinal arteriolar saturation was 93±4% (n=31, mean±SD) in healthy subjects and 101±6% (n=28) in patients with DR (p<0.0001). Retinal venular saturation was 58±6% in healthy subjects and 67±8% in diabetic patients (p<0.0001). Arteriolar and venular saturation was higher in all subgroups of diabetic patients (see methods) than in healthy subjects.

Conclusion Increased oxygen saturation in retinal vessels in diabetic retinopathy, also found by other researchers, is consistent with poor distribution of blood and oxygen to the retinal tissue rather than decreased total retinal blood flow. Poor distribution of oxygen may be caused by capillary dropouts and shunts as well as thickening of the capillary walls.

Commercial interest

■ 2463

Retinal and optic nerve oxygenation and carbonic anhydrase inhibition

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Purpose To study the effects of carbonic anhydrase inhibition on porcine retinal and optic nerve oxygenation under physiological conditions and in experimental models of ischemia.

Methods Polarographic oxygen electrodes were used to measure the oxygen tension in the vitreous 500 microns in front of the optic nerve and retina. Retinal ischemia was produced by diathermia of the superior arcade vein, producing a branch retinal vein occlusion, BRVO. Optic nerve ischemia was produced by intravenous administration of 100 mg Indomethacin intravenously.

Results One week after induction of BRVO, the oxygen tension over BRVO affected retina was significantly decreased by 29%. Administration of the carbonic anhydrase inhibitor dorzolamide (500 mg) caused a significant increase in the oxygen tension over BRVO affected retina, and in effect restored this tension to normal values (n=5). Intravenous administration of 300 mg Indomethacin caused a decrease of optic nerve oxygen tension by 41%. Subsequent administration of 500 mg dorzolamide increased the optic nerve oxygen tension, albeit not to normal levels (n=6).

Conclusion Carbonic anhydrase inhibition increases the oxygen tension in the retina and optic nerve. In BRVO affected retina, carbonic anhydrase inhibition restores oxygen tension to normal levels.

■ 2462

Retinal photocoagulation and oxygenation

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Purpose The clinical role of photocoagulation for the treatment of hypoxia related complications of retinal ischemic microangiopathies is well established.

Methods Measurements of the partial pressure of oxygen (PO₂) distribution within the the retina in various animal species using oxygen sensitive microelectrodes and evaluation of the retinal vessels reactivity by laser doppler velocimetry gave additional insights concerning photocoagulation mechanisms.

Results The PO₂ within the vitreo-retinal interface is heterogeneous. Preretinal and trans-retinal PO₂ profiles indicate that the preretinal PO₂ far away from vessels remain constant in all retinal areas. Intervascular intraretinal PO₂ gradually decreases from both the vitreo-retinal interface and the choroid towards the mid-retina. Close to the pigment epithelium, it is significantly higher than at the vitreoretinal interface due to the much higher O₂ supply provided by choroidal compaires to retinal circulation. Laser photocoagulation reduces the outer retina O₂ consumption and allows O₂ diffusion into the inner retina from the choroid raising the PO₂ in the inner healthy retinal layers and in the preretinal intervacular normal areas. In this way laser treatment relieves retinal hypoxia in experimental branch vein occlusion (BRVO). In patients with diabetic retinopathy (DR), the retinal PO₂ is higher in areas previously treated with laser. Following photocoagulation, the resulting reversal of hypoxia, the retinal vasculature constriction and the improvement of the regulatory response to hyperoxia all affect favorably both the retinal neovascularisation and macular edema.

Conclusion Photocoagulation induces an increase of the inner retinal oxygenation reversing the retinal hypoxia and improving the regulatory response of the retinal vessels

■ 2464

Noninvasive oximetry and glaucoma

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Purpose To investigate retinal vessel oxygen saturation in relation to glaucomatous visual field damage. Specifically, we examined whether oxygen saturation in retinal blood vessels differs between regions corresponding to glaucomatous visual field defects compared to regions without visual field defects.

Methods A spectrophotometric retinal oximeter (Oxymap ehf, Reykjavik, Iceland) was used to measure oxygen saturation in retinal arterioles and venules. The oximeter consists of a fundus camera, beam splitter, light filters and software that evaluate the oxygen saturation. The glaucomatous defect was estimated from a visual field test using the Octopus 1-2-3 perimeter. One eye in 13 individuals with open angle glaucoma with or without pseudoexfoliation syndrome was examined.

Results In retinal areas with no visual field defect, the mean oxygen saturation in arterioles was 102±6% and 65±9%, (mean±SD) in venules. The arteriovenous difference was 37±10%. In retinal areas corresponding to visual field defects, the mean oxygen saturation in arterioles was significantly lower; 98±5% (p=0.04, paired t-test, n=13). The venules were at 68±7% (p=0.3) and the arteriovenous difference was also significantly lower; 30±10% (p=0.04).

Conclusion Arteriolar oxygen saturation and arteriovenous difference is statistically lower in areas with visual field defects compared to areas without visual field defects. This data suggests that visual field defects are associated with a reduction in retinal oxygen delivery and metabolism.

■ 2465

Retinal vessel oximetry made simple and reliable - principle, performance and clinical results

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Purpose The regulation of the oxygen supply to the retinal tissue is critical and the detection of alterations in the oxygen supply or consumption may have a diagnostic merit. Quantitative assessment of oxygen supply and oxygen utilization in the tissue requires the measurement of the blood flow as well as the blood oxygenation. While the blood flow can be calculated from laser Doppler velocimetry and the vessel diameter, the intra-vascular hemoglobin oxygen saturation (SO₂) can be determined by a photometric measurement.

Methods In the current approach, the SO₂ is measured by a dual – wavelength technique. Using a fundus camera, equipped with a special dual wavelength transmission filter and a color CCD camera, two monochromatic fundus images at 548 nm and 610 nm were recorded simultaneously. The optical densities of retinal vessels for both wavelengths and their ratio, which is known to be proportional to the oxygen saturation, were calculated. Measurements of SO₂ were performed in patients with diabetic retinopathy, arterial occlusion, and healthy controls. Furthermore, the changes of SO₂ upon flicker light stimulation of the eye were investigated.

Results From a healthy control population, mean arterial and venous SO₂ were measured to be 98±10.1% and 65±11.7% with reproducibility of 2.52% and 3.25% respectively. In 10 patients with central or branch vein occlusion, a reduction of the arterial SO₂ to 62±11% was found in the occluded vessel. After 5 days on pentoxifylin therapy, the SO₂ increased to 90±16%. Diabetics showed venous SO₂ increasing with the severity of the retinopathy. Flicker increased the venous SO₂ by about 5%.

Conclusion Dual wavelength oximetry is a simple and reliable method for physiological and clinical investigations.

Commercial interest

■ 2466

Retinal vessel oximetry using sequential and 'snapshot' hyperspectral imaging

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Purpose Use of sequential, and 'snapshot' hyperspectral imagers to measure oxygen saturation in retinal vessels in normals, and examples of eye disease, eg glaucoma, and retinovascular diseases. Validation of estimated oximetry values using a model eye.

Methods A sequential hyperspectral imager was constructed using a fundus camera with built-in liquid-crystal tuneable filter. Images of normals, and ocular disease are presented. A novel 'snapshot' hyperspectral imager is also described: this produces images in a single exposure. Validation of both devices using an artificial eye with capillary tubes containing human blood of known oxygen saturation, placed in front of an 'artificial retina' is described. The image analysis used to detect retinal vessels, and generate oximetric values is detailed.

Results Both the sequential, and 'snapshot' retinal imagers produced accurate estimations of retinal vessel oxygen saturation, when compared with the model eye. Imaging of a small group of glaucoma eyes showed abnormally elevated venous oxygen saturation. In proliferative diabetic retinopathy, abnormally elevated venular saturation was found in areas of capillary loss on FFA. In vein occlusion, elevated venous saturation was found in eyes with ischaemic FFAs.

Conclusion Both the sequential and 'snapshot' hyperspectral imagers deliver useful oximetric maps of the retina. The 'snapshot' device allows more rapid imaging. The elevated venular oxygen saturation seen in both glaucoma, and retinovascular disease, is perhaps evidence of reduced oxygen consumption in damaged inner retina in glaucoma, and/or vascular 'shunting' in retinovascular disease.

■ **2471**
Need for training the trainers programme in harmonisation of education

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Purpose Brief introduction to this session will be given to outline the need for structured teaching activities that would focus on the contents (i.e. what to teach) and the teaching methodologies (i.e. how to teach) and methodologies of assessment of knowledge.

Methods The mission of European Board of Ophthalmology is to harmonise training in Europe and the traditional scope of its activities include curriculum development, assessments (EBO Diploma examination), accreditation of training centers, exchange of residents and staff members and CME.

Results "Training the trainers" programmes are recognised as one of important and rapidly developing disciplines, needed to optimise and harmonise the teaching processes, both in medical and surgical domains as well as in improving professionalism.

Conclusion EBO has recognised the potential of TTT courses to advance and harmonise training in ophthalmology and will continue to foster and implement this knowledge to everyday teaching practices.

■ **2473**
Needs from residents' perspective and SOE YO

KHAWAJA A
London

A resident's training needs are very diverse and include acquiring knowledge to pass exams, clinical skills, surgical skills and business acumen. Modern training programs aim to deliver each of these requirements in a more structured and reproducible format. However, not all these needs are amenable to structure. These challenges will be discussed and the recently introduced training scheme in the UK will be highlighted as an example.

■ **2472**
The role of the professor as leader and promotor of science

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Purpose To present essential aspects of the role of the professor as promotor and leader of science

Methods Key elements are presented and illustrated by examples: The creative scientific milieu The typical flow of a young talent in Copenhagen Creating the team spirit Internal courses: Fund raising – list of good funds Statistics How to prepare an article Internal social relationships: Journal club The "professors Court" or the "gossip club" Scientific lines - Buffer funds

Results The effect is a high number of completed PhD projects per professor

Conclusion When you share ideas, dreams, knowledge - like with love, you do not lose any part of it - you gain

■ **2474**
EBO curriculum development

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Purpose To explain the aim of a curriculum development in European Board of Ophthalmology (EBO)

Methods The aim of the European Board of Ophthalmology Diploma (EBOD) is to define a test of excellence in Ophthalmology designed to assess the knowledge and clinical skills requisite to the delivery of homogenous and high standard of Ophthalmology care in hospital. The curriculum workgroup belonging to the Education committee of the EBO aims to define a curriculum to help students during their training. It should provide students with a comprehensive uniformed list of topics of knowledge to stimulate students according to their different levels of training to follow their acquisition. It should be a guide for the students to share their time between different topics. Finally it should define good resources in relation with the curriculum considered as a "European dreamy library" designed for all ophthalmologists.

Results Two steps were defined: one syllabus made of an exhaustive list of items with a list of resources to help the student to find good references. It should be useful for written exam to help students to define the topics to be known with an exhaustive point of view. The second one based on more detailed objectives is based on 3 different levels of objectives: the first one will define the pure knowledge (it is mainly dedicated to epidemiology, pathology, signs, risk factors, genetics ...), the second one will be more advanced as it is dedicated to know how the student will... do something, interpret an exam, explain to the patient... The third one will provide the student with an appropriated list of references in relation with this specific topic.

Conclusion The development of curriculum is a key role of EBO to define the objectives of learning and acquisition during training.

■ 2475

How to conduct a good viva voce?

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The Viva Voce exam is a very useful way of assessing candidates applied knowledge and their decision making process. It is crucial that examiners maximize the use of the limited time allocated in order to be able to make a fair assessment. Guidance will be given on exam conduction, time utilization and questioning technique. Advice will be given on fair marking and the importance of comprehensive completion of the score sheets.

■ 2476

How to teach residents to teach and to select proper residents for this goal

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Purpose It is very important to promote the development of the academic ophthalmologist. Part of the curriculum of residency program should be designed for training in the academic sphere.

Methods Some residents demonstrate verbal and personal characteristics which show an ability to teach. Apart from professional knowledge and skills they possess the aptitude to communicate and emotionally involve the listener. The ophthalmic educators should actively seek out such trainees, focus on them, analyze and improve their case presentations and clinical reports.

Results It is advisable to include these individuals in medical student education from the beginning of their residency. In the late phase of their education the level of responsibility can gradually be increased to teaching new residents and taking part in post-graduate programs. Throughout their ophthalmic residency they have to be continuously encouraged to pursue academic realization. An important aspect of this stimulation should be regular participation in various ophthalmologic events – both nationally and abroad.

Conclusion It is essential to motivate the selected trainees to read journals so as to keep track of new developments in ophthalmology, medicine and public health. The next most important step is active involvement in the process of manuscript creation and publication. Exposure to excellency in education through short visits to accredited training centers and participation in "training for trainers" symposia would be beneficial for understanding the value of good teaching.

■ 2477

Professionalism and appraisal

DUA HS

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Conclusion Professionalism is defined as the expertness characteristic of a professional person. A true professional encompasses attributes of integrity, honesty, transparency, fairness, trust, respect, dignity, courtesy and ethical behaviour, working within means with insight into limitations, updating knowledge & skills, communicating effectively and others. Professionalism also involves striking a balance between conflicting pressures such as work and life, family and colleagues and saying "yes" and "no". In short, professionalism is the ability to put all the above together. Appraisal is a two way process in which the appraiser and appraisee together, in a non-confrontational manner identify the needs of the appraisee, establish the means of fulfilling those needs and set objectives and timeframes in which to achieve those needs. Achievements are recognised and appreciated, problems and their causes where possible are identified and means of solving them are considered and action points agreed. Appraisal should occur regularly. All necessary information should be gathered beforehand, discussed during the appraisal and goals set for the next appraisal. The next cycle should begin by considering the goals set previously and understanding why some were not met and acknowledging those that were. The whole process should be a positive and constructive experience for the appraisee. The individual should have confidence in the appraisal process and look upon it as a process whereby his accomplishments are recognised and where he or she can openly discuss concerns and find answers. A good appraisal benefits the individual and the employing organisation. Unlike 'assessment' it does not pass judgement on the individual's performance but represents a concerted effort to improve performance.

Commercial interest

■ 3111

Surgical experience and outcome of scleral buckling procedures in retinal detachment

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Purpose The purpose of this study was to evaluate the impact of the surgeons' experience on the anatomical and functional outcome of primary scleral buckling surgery in rhegmatogenous retinal detachment.

Methods The charts of patients presenting with a retinal detachment between 2000 and 2006 were analyzed retrospectively. All patients with macular involvement treated with scleral buckling surgery were included. The surgeons were designated according to the "on call" list. Junior surgeons were fellows with less than 2 years of experience, physicians with more than 2 years of experience were defined as senior surgeons.

Results Among the 115 included patients, 76 (65.8%) were operated by senior surgeons. The age, duration of symptoms, initial visual acuity, extension of the retinal detachment were similar in both groups. The primary reattachment rate was 87.7% in the senior surgeon group versus 92.1% in the junior surgeon group ($p=0.36$). In the eyes operated by senior surgeons, the final visual acuity was better than 20/40 in 78.5% versus 63.2% in eyes operated by junior surgeons ($p=0.09$). However, in phakic eyes with limited retinal detachments, the senior surgeons achieved better functional results ($p<0.01$).

Conclusion The surgical experience, except in some subgroups of patients, did not significantly influence the anatomical or the functional overall outcome of patients undergoing primary scleral buckling surgery in retinal detachment with macular involvement.

■ 3113

Sutureless encircling band – biomechanical calculations and clinical experience

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Purpose In cases with multiple retinal breaks and in combination with vitrectomy in eyes with Proliferative vitreoretinopathy (PVR) for retinal detachment surgery often an encircling band is used. Usually the encircling band is fixed with non absorbable sutures.

Methods A fixation method for an encircling band in retinal detachment surgery with one scleral tunnel in every of the 4 quadrants is reported. We describe our experience and biomechanical calculations of this fixation technique.

Results In comparison to conventional fixation technique we found the following advantages: No suture is necessary, this means no additional foreign body can produce irritations. The scleral tunnel is safe and the preparation under the microscope can be performed fast and well controlled. Sclera tunnel fixation is very comfortable in combination with a vitrectomy.

Conclusion With a short learning curve the operating time is as short as with conventional suture fixation of the encircling band. There is less perforating risk, less irritation and less patient discomfort postoperatively. A sutureless encircling band with sclera tunnel fixation is a very usefull operation technique in clinical routine.

■ 3112

Investigation of particular surgical steps in epiretinal prostheses implantation procedure in pigs

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Purpose Proliferative vitreoretinopathy (PVR) is known a known complication of implantation of epiretinal prostheses in porcine eyes using our combined surgical procedure of vitrectomy, lensectomy, large scleral incision and retinal tack insertion. The aim of the present experimental study is to investigate the intraocular reaction to particular parts of the epiretinal prostheses implantation procedure in pigs.

Methods 15 pigs were divided into 3 groups. Group 1 (n=6) underwent vitrectomy, lensectomy, insertion of inactive epiretinal prosthesis through a scleral incision and fixation to the posterior pole with a retinal tack. In group 2 (n=5) vitrectomy, scleral incision and retinal tack insertion were performed. Group 3 (n=4) received vitrectomy, scleral incision and insertion of a shortened prosthesis into the vitreous cavity. The follow up was 4 weeks.

Results PVR was observed in all eyes of group 1 and in one eye of the group 3 with unintentional perforation of the lens capsule by the shortened implant. In all other eyes funduscopy revealed no clinical pathology.

Conclusion Our results indicate that lensectomy is the key stimulus for PVR in porcine eyes while other steps of the implantation procedure are well tolerated. Though pigs do not seem to be a reactive animal model, lens manipulation should be avoided in the surgical procedure for the implantation of retinal prostheses.

■ 3114

Electronic subretinal implants allow blind retinitis pigmentosa patients to read letters and recognize the direction of fine stripe patterns

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Purpose Restoration of letter reading and stripe pattern recognition in blind RP patients by placing subretinal implants transchoroidally near the macula, consisting of two arrays: 4x4 electrodes controlled retroauricularly via a subdermal line for direct stimulation ("DS array") and a "chip" (3x3x0.1 mm), 1500 electrodes.

Methods Letters and stripe pattern were presented to 3 patients via the light sensitive chip – by patterns steadily presented at a screen. On the DS array the sensation evoked by each individual pulse consists of whitish round dot, clearly separated from its neighbor. Patterns consisting of such 4 x 4 dots correspond to letters of approximately 5 cm diameter presented at 60 cm distance.

Results Pat.1 correctly (20/24) recognized the direction of the letter "U", presented with the opening in four different directions (DS array). Pat.2 correctly (12/12) differentiated letters via DS array (e.g. COIL). With the light sensitive chip, he correctly (22/24) differentiated letters (e.g. LITZ; 8.5 cm high, 1.7 cm line width) steadily presented on a screen at 62 cm distance. Pat.3 recognized (15/20 correct, 4AFC) the direction of lines or stripe patterns with the chip, as did Pat.1 (11/14, 2AFC) and Pat.2 (11/12 4AFC) up to 0.35 cycles/deg.

Conclusion Active subretinal multielectrode implants with currents close to produce retinotopically correct patterns that allow for the first time recognition of individual letters and stripe patterns up to 0.35 cycles/deg clearly supporting the feasibility of light sensitive subretinal multi-electrode devices for restoration of useful vision.

■ 3115

First experience with The IRIS retinal implant system

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Purpose To report on the first 4 months experience of a patient with the active IRIS-Implant.

Methods 4 weeks after implantation the training with the active implant started. Thresholds were measured at each training day. Light perception, light localisation, point to point discrimination and motion detection were measured with special test procedures. Visual function training was performed.

Results Visual perception was achieved, when the stimuli were generated by the computer and with the camera mode. All tests were successful.

Conclusion Successful stimulation and major improvements during the training demonstrates that with the Iris Implant System a visual perception can be achieved, which is relevant for daily life.

■ 3121

The study of chromatic and achromatic VEP in the first year of life

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Purpose To study chromatic and achromatic VEP responses in the first year of life.

Methods In 30 babies aged 2 to 12 months VEP to chromatic and achromatic stimuli were binocularly recorded. Chromatic VEP were recorded to isoluminant red-green and blue-yellow stimulus. The stimulus was a circle composed of horizontal sinusoidal gratings with 90 % chromatic contrast. Two stimulus sizes (7 deg and 21 deg) were used. The stimulus was presented in an onset-offset mode (on – 300ms, off – 700 ms). Achromatic VEP were recorded to black and white checkerboard (50' square size), which was presented in pattern reversal (reversal VEP) and onset-offset (onset VEP) mode. VEP were recorded from Oz (mid occipital) position and the reference was at Fz.

Results Chromatic VEP responses were present in all babies, except youngest two (2 months old). The positive wave (P) amplitude to red-green and blue-yellow stimulus increased with age when using both stimulus sizes. Achromatic VEP responses were present in all babies. P100 wave (pattern reversal stimulation) showed longer latency in youngest babies ($p=0.045$), whereas its amplitude did not change throughout the first year of life. C1 wave (onset-offset stimulation) showed longer latency in youngest babies ($p=0.0039$), whereas its amplitude decreased with increasing age ($p=0.0087$).

Conclusion Chromatic VEP responses can be recorded in babies after the age of 3 months and show marked maturational changes throughout the first year of life.

■ 3123

Differential effects of ageing on foveal and peripheral colour vision

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Purpose Colour sensitivity was assessed to establish aging effects both at the fovea and 6 deg away from fixation, in each of the four quadrants.

Methods 65 normal healthy subjects (from 20 to 80 years of age) took part in the study. All subjects had Visual Acuity (VA) of 6/6 or better. Fixation accuracy was monitored using infrared imaging of the pupil and the tests were carried out on the P_SCAN system. Target size was adjusted for parafoveal locations to account for retinal and cortical magnification. Yellow-blue (YB) and red-green (RG) colour discrimination was assessed using the CAD (colour assessment and diagnosis) test (<http://www.caa.co.uk/docs/33/200904.pdf>).

Results RG and YB colour thresholds were analysed separately for all five locations tested and showed no significant effect with ageing below the age of 60 years. Two age bands were formed based on statistical analysis (20-59.9 and 60-79.9). The decline in performance with age was more rapid at the fovea and exhibited a steeper gradient when compared with results in the periphery for both RG and YB discrimination. Foveal YB discrimination showed the largest ageing effect. No significant difference was found between the four parafoveal locations. YB discrimination at the fovea also exhibited the largest inter-subject variability.

Conclusion These findings may have clinical significance in the very early detection of disease processes that remain subclinical in many subjects. Differences between foveal and peripheral locations help to differentiate between the normal effects of ageing and disease. For example, higher foveal and normal peripheral YB thresholds in normal subjects from high peripheral thresholds in early glaucomatous subjects.

■ 3122

Acquired loss of chromatic sensitivity

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Purpose A range of ophthalmic and neurological conditions cause diminished visual performance, even when the subject is often unaware of any problems and the loss of vision remains undetected in conventional perimetry and visual acuity tests. The extent to which detection of acquired colour vision loss can be revealed in subclinical cases and distinguished from congenital loss has been investigated.

Methods Over 400 subjects with congenital and acquired colour vision loss have been examined using conventional colour screening methods. In addition, the loss of yellow / blue and red / green chromatic sensitivity has been quantified using the CAD test (<http://www.caa.co.uk/docs/33/200904.pdf>). Those investigated included subjects with diseases of the retina and / or the optic nerve as well as patients with selective damage to central visual pathways. Patients with various stages of glaucoma, photoreceptor dystrophies, diabetes, optic neuritis, age-related macular degeneration as well as tobacco and alcohol toxicity have been examined.

Results Algorithms developed for analysis of colour vision loss and automatic classification of congenital and / or acquired colour deficiency will be described. In acquired deficiency, the loss of chromatic sensitivity tends to affect both the rg and the yb channels. Significant differential effects have, however, been observed in relation to stimulus size, retinal location and state of light adaptation.

Conclusion The findings from these studies show that in the majority of these conditions, the loss of chromatic sensitivity is the most sensitive measure of early changes in diseases of the eye.

■ 3124

Chromatic sensitivity in subjects with diabetes

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Purpose Diabetic retinopathy is a major cause of blindness in the Western World and remains one of the most serious complications of diabetes mellitus. The gold standard to measure functional change in diabetic (DB) patients is LogMAR or Snellen VA. The aim of this study was to measure and characterise the severity of visual function loss in subjects with DB using sensitive psychophysical tests and to quantify accurately how changes in management of the disease correlate with changes in visual function.

Methods Three groups of patients ($n=50$) were included in this study: DB patients with and without retinopathy and patients with no DB. Each patient had a full ophthalmic examination prior to psychophysical assessment. Colour vision assessment was carried out using the CAD test (Colour Assessment & Diagnosis) that provides a measure of both yellow-blue and red-green loss of chromatic sensitivity (CS). Rapid flicker (RF) was also measured by assessing sensitivity to a 20Hz flicker stimulus at five locations in the central visual field (fovea and 1.5 degrees from fixation in each quadrant). All tests were carried out at photopic and high mesopic light.

Results The results show significant loss of CS ($p < .001$) and some loss in RF sensitivity ($p < .01$). The most sensitive measure of visual loss was CS. There was also a positive correlation between CAD thresholds as a measure of CS and improved DB control.

Conclusion The results suggest that loss of visual function precedes structural changes in the retina and could be used as a means of detecting early structural changes in the retina that precede clinical diagnosis of retinopathy. Preliminary results also suggest that changes in CAD thresholds provide an objective way of monitoring the progress of DB and/or treatment outcome.

■ 3125

Pre-receptor spectral absorption, healthy ageing and pre-clinical indications of retinal disease

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Purpose The aim of this study was to investigate how chromatic sensitivity changes as a function of age and to establish the extent to which such changes can be attributed to pre-receptor spectral absorption of short wavelength light and/or changes in retinal mechanisms caused by ageing.

Methods The absorption of blue light by the macular pigment (MP) and the crystalline lens and the subjects' sensitivity to rapid flicker were measured using the Macula Assessment Profile (MAP) test. Red-green (RG) and yellow blue (YB) chromatic detection thresholds were measured at the fovea for young and older subjects using the Colour Assessment and Diagnosis (CAD) test at 2.6, 26 and 65 cd/m². The variables of interest included the spectral absorption of the lens, the optical density of the MP, subject's age and retinal illuminance.

Results The absorption of blue light by the lens increased with age. Absorption of blue light by pre-receptor filters did not affect RG chromatic sensitivity at any of the light levels investigated but had an effect on YB thresholds. The considerably higher colour vision thresholds of some subjects and the subsequent worsening of their chromatic sensitivity at the lower light level may reflect changes in the retina brought about by accelerated aging effects.

Conclusion The effect of pre-receptor absorption of blue light on chromatic sensitivity is small. Ageing affects the amount and spectral composition of the light reaching the photoreceptors and the processing of retinal signals. As a result, flicker sensitivity declines and colour vision deteriorates. Such effects arise mostly from changes in the retina. The MAP and CAD tests help us to detect the effects of accelerated ageing and retinal disease.

■ 3126

On the effectiveness of cone mosaic geometry in sampling patterns near the Nyquist frequency

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Purpose The cone mosaic is characterised by a quasi-periodic hexagonal organization. The purpose of this work was to develop a mathematical model of cone mosaic including irregularities and to employ this model to evaluate its effectiveness in sampling simple patterns including optotypes (letters) of different sizes and gratings.

Methods Cone mosaic images were analyzed using purposely-developed computer programs and a stochastic model of the human cone mosaic was created. The computer-generated mosaics were used to sample sequences of patterns translated to positions randomly extracted from experimental data of fixational eye movements. The sampling efficiency was evaluated as the RMS difference between the initial and the sampled pattern. Similar simulations were performed using a periodic mosaic of equal average cone size.

Results Sampling efficiency (as described by the minimum RMS difference) was better for the quasi-periodic sampling compared to the periodic sampling especially when the sampled pattern involved spatial frequencies higher than 20 cpd. The periodic sampling performed better only in the case where the spatial frequencies in the Fourier spectrum of the sampled pattern exactly matched the spatial frequency associated to the cone spacing (divided by an integer). However the quasi-periodic sampling was more effective in all other pattern sizes and orientations.

Conclusion Although the trichromatic nature of the human cone mosaic as well as the neural organization in the retina after phototransduction were not taken into account, our simulations demonstrate that the geometric irregularities at the cone mosaic may result to more efficient sampling of simple patterns of arbitrary size and orientation.

■ 3131

Pterygium surgery long term follow-up

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Purpose To determine if a pterygium surgical procedure consisting of minimal conjunctival removal, excision of the hypertrophic subconjunctival fibrovascular tissue, application of mitomycin 0.25 mg/ml for 1 minute combined with temporary nasal tarsorrhaphy, and postoperative dexamethasone/antibiotic drops achieved the following: safely simplified pterygium removal, controlled the early side effects of mitomycin, reduced the rate of recurrence, and eliminated the need for conjunctival transplantation.

Methods Twenty eyes of 19 patients underwent the procedure with mitomycin; fifteen were primary and 5, recurrent. These were compared to a previous group of 28 eyes in 26 patients that underwent pterygium/tarsorrhaphy surgery without mitomycin; twenty had primary and 8 had recurrent pterygia. Postoperatively, all eyes in both groups were treated with dexamethasone/antibiotic drops.

Results In the mitomycin group (average follow-up 25 months), 19 of 20 eyes healed uneventfully. At 12 months, there had been no recurrences in the mitomycin group. In the non-mitomycin group (average follow-up 43 months), 9 (32%) recurred within 12 months; four (44%) of these required a second procedure at an average of 9 months. At 23 and 33 months, 2 (10%) eyes treated with mitomycin presented with asymptomatic, one mm recurrences that required no additional treatment. Conjunctival healing, as reflected in the time from surgery until tarsorrhaphy opening, was significantly longer in the mitomycin group, 37 vs. 17 days ($P = .001$).

Conclusion The described technique provided a safe and successful approach to pterygium management.

■ 3133

Detection of osmoprotective effect of topical compatible solutes on ocular surface epithelia

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Purpose Inflammation and hyperosmolarity play an important role in generating dry-eye related damage on the ocular surface. A new approach is the use of topical compatible solutes (CS) to induce osmoprotective effect on ocular surface epithelia. Aim of this study was to evaluate the osmoprotective action of topical CS on ocular surface in dry-eye patients.

Methods We evaluated 48 eyes of 24 dry-eye patients, current users of artificial tears. At baseline they underwent impression cytology (IC) and immunofluorescence staining to detect expression of inflammatory cytokines linked with hyperosmolarity in ocular surface epithelia. In vivo confocal microscopy (IVCM) of corneal and conjunctival surface was performed and epithelial morphology and inflammatory cells infiltration were evaluated. Patients started topical treatment with methylcellulose and CS in one randomly selected eye, while the fellow eye continued previous eye drops. IC and IVCM were performed after 30, 60 and 90 days.

Results At baseline IVCM and IC results were comparable in both eyes. At 90 days IVCM showed a significant improvement in epithelial regularity in treated eyes while in control group no significant changes were observed. IC evidenced a significant reduction of inflammatory cytokines between baseline time and day 60, stable till the end of follow-up, while in control group no significant changes were observed.

Conclusion The use of topical CS can improve epithelial regularity and reduce ocular surface inflammation in dry-eye syndrome.

■ 3132

Distribution of amyloid and BIG-H3 in the cornea and limbus of a patient with lattice corneal dystrophy. Unique findings in donated eyes

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Purpose The lattice corneal dystrophies (LCDs) are hereditary diseases involving the formation of opaque or refractile, amyloid-containing filaments in the corneal stroma. We report the distribution of amyloid and big-h3 protein in cornea and limbus in a patient suffering with LCD.

Methods An 84 year-old patient with lattice corneal dystrophy died and donated her eyes for further study. The corneal and limbal tissue of the patient processed for light and electron microscopy. The primary polyclonal antibody big-h3 was located by secondary, goat anti-rabbit antibody conjugated with gold.

Results In cornea amyloid deposits were observed below epithelium, and in the anterior and middle stroma. The epithelium was thin and invaginated by the amyloid deposits. In the limbus, large numbers of amyloid deposits were observed in sub-epithelial region, and in the mid and deep stroma. Subepithelial amyloid was also present in the substantia propria beneath the bulbar conjunctival epithelium. The amyloid deposits contained very thin amyloid fibrils and strongly stained with big-h3 antibody. There were also numerous long spacing collagen fibrils observed in the mid stroma, which also labelled with the antibody.

Conclusion This is the first report of structural changes in the peripheral cornea and limbus in LCD. It is thought that mutated big-h3 protein diffuse into the stroma from the corneal epithelium to form amyloid deposits. The presence of amyloid and big-h3 at the limbus and in the adjacent bulbar conjunctiva and perilimbal cornea, suggests that Big-h3 is overproduced in these regions, which are normally free from clinically detectable disease.

■ 3134

Alcohol vs. mechanical delamination in the treatment of corneal erosion: an electron microscopic study

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Purpose To performed an electron microscopy study to investigate the cleavage plane and the efficacy of alcohol delamination in recurrent corneal erosion (RCE).

Methods By electron microscopy we analysed the epithelium of: seven controls treated with mechanical debridement, seven controls treated with alcohol delamination, ten cases of traumatic RCE due to MDFFP treated with alcohol delamination, with special regard to the epithelial cells and the cleavage plane. Moreover we analysed four corneas from penetrating keratoplasty that were treated by alcohol delamination on the bench and both the epithelium and stroma were studied.

Results In traumatic RCE the basement membrane remained in situ, a precondition for quick epithelial healing. In MDFFP the whole basement membrane was detached from the stroma and remained adherent to the epithelium, therefore after alcohol delamination the healing process should be different between MDFFP and traumatic RCE.

Conclusion The present findings give strength to alcohol delamination as a promising treatment for RCE.

■ 3135 / 301

Lymphocyte and markers of inflammation detection in the conjunctival epithelium of patients with dry eye by enhanced flow cytometry

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Purpose The aim of our study was to test the hypothesis that the use of cell culture medium can increase the number of cells obtained by impression cytology (IC) sampling of the conjunctiva in dry eye patients, and make the analysis of epithelial and lymphocyte cell populations with flow cytometry possible.

Methods IC specimens were collected in 15 normal subject and 15 dry eye patients. Samples collected from the right eye were placed in cell culture medium containing 10% foetal calf serum (FCS), and samples from the left eye in Phosphate Buffered Saline containing 0.05% paraformaldehyde (PFA). The number of cells was analyzed by flow cytometry in both groups. Samples collected from a group of 30 dry eye patients were placed in FCS and stained for the expression of CK19, CD45, CD3, HLA-DR, and analyzed by flow cytometry. A control group of 10 subjects was used as control.

Results The number of cells counted in the FCS samples was statistically increased in the normal (12791 ± 11350 events per minute) and dry eye group (23468 ± 15596) when compared to PFA samples (2031 ± 2336 , and 2608 ± 1814 respectively). In dry eyes the low number of cells in PFA samples made possible only the analysis of HLA-DR expression, while in FCS samples epithelial (CK19+), leukocyte (CD45+), and lymphocyte (CD3+) cell populations were characterized, other than analyzed for HLA-DR expression.

Conclusion This study indicates that using this new method of preservation can enhance flow cytometry analysis of epithelial and immune cells of the conjunctiva in dry eye patients, opening new scenarios in the comprehension of its pathogenesis and in testing new therapies.

■ 3137 / 303

Ocular surface findings in patients with congenital aniridia

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Purpose to study the Schirmer's test, tear Break-up time, ocular tear ferning pattern test and impression cytology results in patients with congenital aniridia

Methods 25 eyes of 25 patients with congenital aniridia underwent Schirmer's test I, tear break-up time, ocular ferning pattern test and impression cytology to study the ocular surface characteristics. Clinical findings were correlated with the results of the aforementioned tests.

Results Aniridia related keratopathy was Grade 0 in 12%, Grade 1-A in 52%, 1-B in 20% and Grade 2 in 16% of eyes studied. Schirmers Test I was normal in 96% of eyes. Tear break-up time was abnormal in 75% of patients. Ocular ferning pattern tests were as follows: Grade 1 (20%), grade 2 (50%), Grade 3 (20%) and Grade 4 (10%). Conjunctival squamous metaplasia revealed: Grade 0 (23%), Grade 3 (35%), Grade 5 (4%). Conjunctival keratinization was mild in 35% and moderate in 15%. Significant correlation was found between conjunctival keratinization and the degree of Aniridia-related keratopathy. Significant correlation was also found between ocular ferning pattern test and conjunctival keratinization.

Conclusion Aniridia-related keratopathy is a muco-deficient and lipo-deficient, more than an aqueo-deficient dry eye disease. Simple tests of the ocular surface must be done early on to direct the right kind of dry eye treatment in these difficult cases.

■ 3136 / 302

Sutureless and glue free conjunctival autograft in pterygium surgery

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Purpose To review the safety and efficacy of sutureless and glue free conjunctival autografting for the treatment of primary pterygium

Methods Retrospective case notes review of 8 consecutive patients undergoing primary pterygium excision performed by a single surgeon over a 1 year period. Excision of the pterygium was followed by reconstruction using suture and glue free conjunctival autograft.

Results Mean follow up was 14 months (range 12 to 24). There were no transplant dislocations, failures or recurrences (0 of 8 eyes) after one year. The average operating time was 14 ± 2 (SD) minutes. One patient had transient graft oedema. Visual acuity was unaltered in seven patients, one patient with significant pterygium-induced astigmatism gained 3 Snellen lines. No major complications occurred and all patients approved of their cosmesis at their final visit.

Conclusion Sutureless and glue free autologous conjunctival grafting for primary pterygium surgery appears safe and efficacious and may aid in preventing complications attributed to the use of foreign materials.

■ 3138 / 304

Compare optical with photometry measures of tear film lipid layer

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Purpose Quantity of tear film lipids could be estimated optically by colours of interference. By photometric evaluation it may be measured. Therefore we compared both measures.

Methods Patients with dry eyes were observed by Tearscope and Meibometer 550. For tearscope we used the 4-parted scale of colours. Meibometry was performed with clean stripes, which have touched the tear film. >300 units are said to be normal. We observed the tear film by Schirmer I, Lissamin green and fluorescein staining, and the break up time. We correlated the measurements with Pearson Correlation.

Results 85 eyes of 53 patients were observed. Both measurements of the lipids showed positive correlations $R=67$. There was no Correlation between any lipid measurement and the watery and mucin Layer ($R=0,1 - 0,3$)

Conclusion The measurement of lipids is similar in both measurements. The optical evaluation needs longer experience, the Meibometry needs standards to find reproducible results. The lack of correlation between lipid layer results and the other results of the tear film observation may point at the special function of lipids independent of the other layers of the tear film.

■ 3139 / 305

Evaluation of spatial contrast sensitivity after the instillation of diclofenac eye drops

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Purpose To evaluate if diclofenac eye drop instillation is related with spatial contrast sensitivity (CS) impairment.

Methods Thirty ophthalmologically healthy Caucasian individuals (--male, --female), aged from 20 to 59 underwent CS testing. The examination was repeated 20 and 40 minutes after the instillation of diclofenac eye drops unilaterally. The fellow eye served as control.

Results All the examined individuals had normal visual acuity, color vision and CS before the diclofenac drop instillation. Four of them complained of a temporary glare at the eye in which diclofenac was instilled. These four individuals had decreased CS in low spatial frequencies (1.5 & 3 cycles/degree), in the examination performed 20 minutes after the instillation. The CS normalized again in the third CS evaluation performed 40 minutes after the instillation.

Conclusion The temporary glare that affects visual performance of some individuals after diclofenac eye drop instillation is related with a temporary decrease of spatial CS in low frequencies. Within this time period of 40 minutes after the instillation of diclofenac, individuals who experience visual disturbances should avoid activities that demand high visual efficacy or postpone the instillation for a more convenient time in relation to the duration of glare they have experienced.

■ 3141

Loss of thioredoxin activity as an important contributor to oxidative stress in lens of diabetic rats

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Purpose Thioredoxin (Trx) is a redox regulator whose bioavailability in mammalian tissues is regulated by its intrinsic binding protein, thioredoxin-binding protein-2 (TBP-2). The purpose of this study is to investigate whether diabetic condition can alter the ratio of Trx and TBP-2 expressions in the lens that may contribute to cataract formation.

Methods Streptozotocin (STZ at 75 mg/kg) induced diabetic rats were divided into aldose reductase inhibitor (0.0125% AL1576) treated and untreated groups. Rats without STZ injection were used as controls. Lenses from 3 and 8 wks post STZ injection were used to determine GSH level, Trx activity, and Western blot analysis for Trx and TBP-2 protein levels.

Results The lenses in the diabetic untreated group showed slight haziness in 3 wks, and moderate to severe opacity in 8 wks while all lenses in the ARI-treated or control groups remained clear in both ages. The untreated diabetic group showed severe loss in GSH (< 20% of control in both 3 and 8 wks) and Trx activity (< 35% of the control in both 3 and 8 wks). TBP-2 expression although was unchanged at 3 wks but elevated ~4-fold over the control in 8 wks while Trx expression remained constant at both time points. ARI treatment protected the GSH level, Trx activity, and TBP-2 expression in these lenses.

Conclusion Diabetic condition induced TBP-2 expression in the lens with concurrent suppression of Trx activity. The results suggest that the loss of the bioavailability of TRx, in conjunction with a severe GSH depletion, is likely to contribute to the oxidative stress observed in diabetic conditions. Furthermore the observation that ARI can normalize these changes suggests a role of osmotic stress in Trx/TBP-2 imbalance.

■ 3143

Anticataract effect as a predictor of drug efficacy against early diabetes-induced retinal changes

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Purpose To evaluate if anticataract effect predicts drug efficacy against early diabetes-induced retinal changes, using STZ-diabetic rat model and cultured retinal microvascular cells.

Methods Mature control and STZ-diabetic rats received one of the following treatments (a prevention approach): 1) the aldose reductase inhibitor (ARI) fidarestat, 2) the PARP inhibitors, 1,5-isoquinolinediol or GPI-15427; 3) the Na⁺/H⁺-exchanger-1 (NHE-1) inhibitor cariporide; and 4) two agents, counteracting endoplasmic reticulum stress, trimethylamine N-oxide dehydrate or 4-phenylbutyric acid. The duration of diabetes was 12 wks. Cataract formation was monitored by indirect ophthalmoscopy and slit lamp examination. Oxidative stress and apoptosis were assessed as early diabetes-induced retinal changes in rat retinae and cultured bovine retinal pericytes and endothelial cells.

Results An ARI treatment completely prevented cataract formation in STZ-diabetic rats, whereas three other treatments delayed, but did not prevent, diabetes-induced cataractogenesis. All four classes of agents counteracted diabetes-induced apoptosis in rat retinae, and the ARI, PARP inhibitor, and NHE-1 inhibitor treatments prevented high glucose and NEFA-induced apoptosis in pericytes and endothelial cells. Fidarestat, PARP inhibitors, and cariporide treatments also counteracted diabetes-related oxidative stress in the retinae and cultured retinal microvascular cells. The effect of agents counteracting endoplasmic reticulum stress on retinal oxidative stress has not been explored.

Conclusion Anticataract effect is a likely predictor of the efficacy of experimental pharmacological agents against, at least, early diabetes-associated retinal changes which may facilitate diabetes drug discovery.

Commercial interest

■ 3142

Treating diabetic dogs with topical Kinostat™ for cataracts: proof of concept clinical trial

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Diabetic dogs rapidly form bilateral sugar cataracts within one year of diagnosis. Similar sugar cataracts also form in galactose-fed dogs. Since topical Kinostat™ has been shown to reverse initial cataract formation in young galactose-fed dogs, we have conducted a proof of concept masked pilot study with 41 newly diagnosed dogs with diabetes mellitus (DM) to investigate whether Kinostat™ can inhibit the progression of cataracts in a clinical setting. After obtaining owner consent to participate in the study, all dogs were randomly assigned a coded vial containing either Kinostat™ or vehicle for 1 year with the contents of the vial (drug or placebo) masked from the examiners. Thirty dogs received the active agent while the remainder received the placebo vehicle. Owners were instructed to administer the agent 3 times daily to both eyes and compliance was monitored by recording each time of administration. Ocular examinations on dilated eyes conducted at the initial examination and at 1, 2, 3, 6, and 9 month intervals with slit lamp and indirect ophthalmoscopy indicated that Kinostat™ is beneficial in arresting the onset and/or progression of cataracts in dogs with DM. The implications of this study for human sugar cataracts will also be discussed. Supported by NIH SBIR 1R43EY018013-01A1

Commercial interest

■ 3144

Differences in lens opacification in type 2 diabetic patients on oral anti-diabetic medication or insulin therapy

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Purpose Evaluation of the effects of different anti-diabetic therapies on light scattering in the lenses of type II diabetic patients compared to data from age-matched normal patients.

Methods The anterior eye segment of 53 patients (20 males and 33 females, mean age 68,23 years) from a private practice, 25 of which were on insulin therapy and 28 on oral anti-diabetic therapies, were photographed with a Topcon SL-45 Scheimpflug camera on Kodak Tmax 400. After standard development the images were digitized and evaluated in respect to density in defined lens layers. Statistical comparison was performed with regression analysis between diabetics and normal patients for 9 defined lens layers.

Results Density data in lens layers 1 – 3 and 5 – 8 did not differ between the 2 treatment groups, whereas in layer 4 (anterior lens cortex) light scattering was significantly lower than in the group of patients on oral anti-diabetic therapy. Gender had no influence on the data evaluated in this study.

Conclusion In contrast to other, larger studies comparing different anti-diabetic therapies, this study shows a trend indicating that insulin might provide a better protection of lens crystallins against non-enzymatic glycosylation than oral anti-diabetic medication. Further studies are needed to differentiate between various oral anti-diabetic drugs.

■ 3151

Preoperative topical non-steroidal anti-inflammatory drug or steroid and clinical outcomes after trabeculectomy

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Purpose To investigate the benefit of preoperative topical non-steroidal anti-inflammatory drug (NSAID) or steroid after trabeculectomy.

Methods In this prospective randomized placebo-controlled trial, 61 patients scheduled for trabeculectomy were randomized to one of 3 study medication groups: NSAID (ketorolac), steroid (fluorometholone) or placebo (artificial tears). Patients instilled one drop 4x daily for 1 month preoperatively and were examined on day 1, 2, at weeks 1, 2, and 4, and at months 3, 6, 12, 18 and 24 following trabeculectomy. Main outcome measures were incidence of postoperative interventions and IOP-lowering medications; complete and qualified success rate; final IOP and relative IOP reduction.

Results Fifty-four eyes were entered for analysis. The percentage of patients requiring needling within the first year was 41% in the placebo, 6% in the NSAID and 5% in the steroid group ($P = 0.006$). The percentage of patients requiring IOP-lowering medication at one year was 24% in the placebo, 18% in the NSAID and 0% in the steroid group ($P = 0.054$ overall; $P = 0.038$ for steroids versus others). Log-rank test showed a significant ($P = 0.019$) difference in medication-free survival curves between the different groups; patients in the steroid group needed significantly less medication ($P = 0.007$). The inter-group differences in one-year IOP, relative IOP reduction and success rates were not significant.

Conclusion Topical ketorolac or fluorometholone for one month preoperatively was associated with improved trabeculectomy outcomes in terms of likelihood of postoperative needling. Within the steroid group, there was a significant reduced need for postoperative IOP-lowering medication.

■ 3153

Do patients with normal tension glaucoma have a thinner conjunctiva?

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Purpose The central cornea is thinner in patients with normal tension glaucoma (NTG). We had developed the surgical impression of thinner conjunctivas in patients with NTG. The purpose of this study was to determine whether there is a difference between the conjunctival thickness of patients with NTG and those with high tension primary open-angle glaucoma (POAG).

Methods In this prospective study, 40 patients scheduled for trabeculectomy were categorized into NTG and POAG based on maximum intraocular pressure (IOP) as measured by Goldmann applanation tonometry. Ten (10) patients with NTG (max. IOP ≤ 21 mmHg) and 30 patients with high tension POAG (max IOP > 21 mmHg) were included in the study. Conjunctival biopsies taken from the inferior fornix one month prior to trabeculectomy were fixed in formalin and embedded in Histo-resin. The conjunctival thickness was measured on a standardised way and compared between the two groups. Non-paired Student T test for two-tailed groups with equal variance was used for statistical analysis.

Results The difference in mean conjunctival thickness between patients with NTG ($66.4\mu \pm 21.1$) and patients with high tension POAG ($104.6\mu \pm 44.3$) was statistically significant ($P = 0.045$). The mean CCT in NTG (537.6 ± 19.6) was lower than in POAG (548.3 ± 38.0), but did not reach significance in this study.

Conclusion Patients with NTG have a thinner conjunctiva than those with high tension POAG

■ 3152

Microplasmin improves surgical outcome in a rabbit model for trabeculectomy

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Purpose This study was designed to study the efficacy and safety of Microplasmin as an anti-scarring agent after trabeculectomy in a rabbit model.

Methods The effect of Microplasmin was investigated in vivo in a rabbit model for glaucoma surgery. Clinical outcome measures were intra-ocular pressure, bleb area and survival, side effects on slit lamp examination. Moreover, (immuno-) histochemical analysis of the eyes was performed, with quantification of inflammation (CD 45) and collagen deposition (Trichrome and Sirius Red). In the first experiment ($n=10$), Microplasmin anterior chamber injection was compared to placebo injection. In the second experiment ($n=3$), topical Microplasmin drops were compared to placebo drops. In the third experiment ($n=5$) the combination of Microplasmin anterior chamber injection and topical drops was compared to placebo injection and drops. All experiments were conducted in a masked observator way.

Results Microplasmin significantly augmented the bleb area and survival in a rabbit model of trabeculectomy after a single anterior chamber injection or combination therapy (injection combined with drops) compared to control. Collagen deposition was borderline reduced after Microplasmin administration compared to control. No significant changes in inflammation were noticed in the anterior chamber or in the conjunctiva.

Conclusion Microplasmin single injection or combination with postoperative drops improved the outcome after trabeculectomy. In a rabbit model, larger blebs were produced for a longer period compared to control, and collagen deposition tended to decrease in this small series.

■ 3154

Effect of phacoemulsification on the primary open angle glaucoma control after trabeculectomy: a case-control study

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Purpose In this retrospective and comparative study, we analyzed the influence of phacoemulsification (PE) on the glaucoma control in case patients (trabeculectomy-phacoemulsification [CE]) compared with the control that underwent trabeculectomy (T) alone in eyes with primary open angle glaucoma (POAG).

Methods Twenty one patients who underwent PE subsequent to T were identified, and 41 who underwent T alone were matched. Visual acuity (VA), intraocular pressure (IOP), bleb appearance, vertical cup disc ratio (VCDR), visual field (VF), glaucoma medications, iris manipulation and complications were documented. Mean follow up was 12 months. Success was defined when IOP ≤ 21 mmHg with the absence of glaucoma medication and/or further surgical intervention.

Results Patients in CE group had no significant change in IOP from pre-operative measures to 12 months post-operative ($p=0.001$). The mean IOP reduced from 15.3 mm Hg to 14.7 mmHg postoperatively. The control group showed an average IOP reduction of 6 mm Hg in the last visit. In CE group, 19% required 1 or 2 glaucoma medications at one year follow-up vs 19.5% in the control group. In CE group 9.5% showed flattening of previously formed bleb in the last visit ($P < 0.001$), 9.7% ended with flat bleb in the T group. The increase in VCDR for the CE group was statistically significant when compared to the control group ($p < 0.001$). Patients in CE group were more likely to exhibit a change in VF (47.6% versus 7.3% respectively). The study is limited by the small number of cases available.

Conclusion Corneal PE in eyes with filtering blebs does not adversely affect long-term glaucoma control in patients with POAG.

■ 3155

A new technique for diode laser cyclophotocoagulation: short term results

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Purpose To evaluate the efficacy of a new technique for diode laser cyclophotocoagulation in refractory glaucoma.

Methods A consecutive case series of 8 eyes of 7 Caucasian patients who underwent gonioscopy assisted diode laser cyclophotocoagulation (GADC). GADC with a peripheral corneal approach is a new surgical technique that employs a manual gonioscopy, iris hooks, ophthalmic operating microscope and an 810 nm laser diode probe usually utilized for retinal photocoagulation

Results The mean follow-up time was 5.9 months (range 3 to 11 months). Mean intraocular pressure (IOP) (\pm SD) was reduced from 24.5 ± 4.3 mmHg to 11.25 ± 1.7 mmHg. The mean number of IOP lowering eye drops (\pm SD) was reduced from 2.0 ± 0.8 preoperatively to 0.8 ± 0.5 postoperatively. The visual acuity remained unchanged in 7 of 8 eyes (87.5%) and deteriorated in 1 of 8 eyes (12.5%). Early complications included IOP spike in one patient. No major complications were encountered. No eyes required repeat cyclophotocoagulation.

Conclusion Gonioscopy assisted diode laser cyclophotocoagulation with peripheral corneal approach appears to be an effective and safe surgical treatment of refractory glaucoma and has the advantage of no requiring of new endoscopic devices.

■ 3156

Inflammation assessment after selective laser trabeculoplasty (SLT) treatment

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Purpose Glaucoma is a progressive neuropathy, reducing intraocular pressure (IOP) seems to be the only treatment to stop progression in glaucoma. There are several methods to reduce IOP: medical treatment, laser and surgery. Selective Laser Trabeculoplasty (SLT) is a new treatment alternative. SLT selectively targets the pigmented cells of the trabecular meshwork without causing thermal or collateral damage to the surrounding structures. The aim of the present study was to assess inflammation after SLT treatment.

Methods 40 patients (80 eyes) were included in the study. Inclusion criteria: Glaucoma (POAG, pigmentary and pseudoexfoliative glaucoma)/ OHT patients that will be treated with SLT in just one eye, both with and without eye-drops. Exclusion criteria: patients suffering from ocular or systemic inflammatory diseases or treated with cortisone or immunosuppressive drugs. Inflammation was measured in 2 different ways: 1) clinically with a slit lamp and classified 0-4; 2) with a "Laser flare meter (Kowa FM 500)". Measurements were done before, 2 hours after, 1 week and 1 month after SLT treatment, both eyes were evaluated. IOP was also checked in the same way. SLT treatment was performed in 90° with the SLT Solo Ellex laser.

Results inflammation before and after SLT showed no significant difference measured both clinically with slit lamp and objectively with the laser flare meter. No inflammation was found in the untreated eyes. No IOP peaks after SLT treatment were found.

Conclusion SLT treatment seems not to induce inflammation in the anterior chamber when 90° were treated. SLT treatment might be considered as a first choice treatment against high intraocular pressure.

■ 3161

Oculopalatal tremor

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Oculopalatal tremor is a rare but disturbing acquired motor disorder. It is a delayed complication of a brainstem lesion (usually infarction or hemorrhage) interrupting the so-called "Mollaret's triangle" (dentato-olivary pathway). Clinically, patients complain of vertical oscillopsia due to a rhythmical vertical pendular movement of the eyes. Other muscles can be involved, namely soft palate, pharynx, larynx, head, face, diaphragm. Hypertrophy of the inferior olivary nucleus is the hallmark of oculopalatal tremor, by MRI or by pathology. This presentation will review the current knowledge regarding the anatomic bases of this syndrome, as well as its clinical presentations, MRI findings, treatment options and pathophysiologic hypotheses.

■ 3162

Opsoclonus

EGGENBERGER E

East Lansing

Opsoclonus is a rare but important eye sign. It is the most extreme saccadic intrusion, and exists on a scale including ocular flutter. While flutter is horizontal back-to-back saccades without an inter-saccadic interval, opsoclonus is multidirectional back-to-back saccades without an inter-saccadic interval. Opsoclonus is often accompanied by myoclonus ("dancing eyes, dancing feet syndrome"), and may result from paraneoplastic (anti-R1), brainstem encephalitis, parainfectious, toxicity, or other systemic conditions.

■ 3163

Superior oblique myokymia

KAWASAKI A

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Superior oblique myokymia (SOM) is an uncommon disorder characterized by episodic monocular oscillopsia. In most cases of SOM no specific etiology is found but occasional cases have been associated with intracranial tumors and head trauma. Recent radiologic evidence suggests that microvascular compression of the fourth cranial nerve may be a pathogenetic mechanism of SOM. This presentation will review the clinical presentation and course of SOM, including video examples, and discuss treatment options.

Commercial interest

■ 3164

Macrosquare wave jerks

EGGENBERGER E

East Lansing

Macro-square wave jerks (MSWJ) are a specific saccadic intrusion. Both the more common square wave jerks (SWJ) and the rare macrosquare wave jerks are horizontal saccadic intrusions with a normal intersaccadic interval; these two saccadic intrusions differ from each other concerning their size: SWJ are less than 5 degrees, while macrosquare wave jerks are greater than 5 degrees. Occasional SWJ, especially in the older population, may be normal, while the presence of macrosquare wave jerks always indicates pathology (MS, multiple system atrophy have been reported).

■ 3165

Ocular neuromyotonia

BOSCHIA

Ophthalmology, Bruxelles

Ocular Neuromyotonia (ONM) is rare disorder characterized by intermittent diplopia, lasting minutes or seconds, provoked by brief, episodic contraction of muscles supplied by the oculomotor, trochlear, or abducens nerve. These episodes are induced by change in the gaze direction or after holding eccentric gaze. Previous radiation therapy of the sellar or parasellar regions is the most frequent cause. Long standing compression of the oculomotor nerve, by tumor, supraclinoid aneurysms, arachnoiditis, basilar artery dolichoectasia, and others have been reported. Although the exact pathogenesis of ONM is still unknown, there is wide acceptance of the segmental demyelination hypothesis. This presentation will review the clinical presentation, discuss diagnosis work-up as treatment options.

■ **3211**
Hereditary vitreoretinopathies

SNEAD M
Cambridge

ABSTRACT NOT PROVIDED

■ **3212**
Protein screening in vitreous samples of patients with retinal vein occlusion

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Purpose The aim of the study was to identify proteins involved in the pathogenesis of retinopathy after retinal vein occlusion. In retinal vein occlusion, proteins penetrate from leaky vessels into the vitreous. Alternatively, retinal cells produce protein factors and release them into the vitreous.

Methods Vitreous and plasma samples of patients with retinal vein occlusion or macular pucker / macular hole were analyzed by antibody microarrays and ELISA.

Results An antibody based microarray with more than 500 target for screening vitreous samples initially was less enlightening than antibody arrays providing the possibility to quantify up to 30 proteins in an ELISA-like microassay. Standard curves of antibody microarrays are as linear as those of ELISAs. VEGF values were similar to values measured by ELISA.

Conclusion In our screen, we found some candidate factors which are currently investigated for their potential of influencing retinopathy after retinal vein occlusion. The use of microarrays to identify protein factors involved in retinal disease in the vitreous will be discussed.

■ **3213**
Pharmacokinetics of intraocular microplasmin. Relevance to the mechanism of action

DESMET M

ABSTRACT NOT PROVIDED

■ **3214**
Insights into the molecular basis of rhegmatogenous retinal detachment

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Purpose Factors that determine the likelihood of developing posterior vitreous detachment and subsequent rhegmatogenous retinal detachment (RRD) include

- i. the degree of vitreous liquefaction
- ii. the strength of post-basal vitreoretinal adhesion and
- iii. the topology of the posterior border of the vitreous base.

The purpose of these studies was to investigate each of these using a combination of ultrastructural and molecular techniques.

Methods Ultrastructural studies of the human vitreous and vitreoretinal interface were performed in combination with various antibodies and cationic dyes. Biochemical studies were performed on extracted vitreous components.

Results The resultant data suggest that:

- i. vitreous liquefaction is caused by the aggregation of vitreous collagen fibrils and this is due to a loss of type IX collagen proteoglycan from the fibril surfaces;
- ii. interactions between heparan sulphate proteoglycans in the inner limiting lamina and components on the surface of cortical vitreous collagen fibrils contribute to postbasal vitreoretinal adhesion;
- iii. the posterior border of the vitreous base migrates posteriorly with aging due to the synthesis of new vitreous collagen by the peripheral retina.

Conclusion The molecular basis of RRD is starting to be unravelled. Furthering our understanding of the underlying molecular processes may lead to the development of novel therapeutic strategies to treat RRD and other vitreoretinal disorders.

■ 3215

Transthyretin levels in the vitreous correlate with change in visual acuity after vitrectomy

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Purpose Little is known about biochemical markers related to change in visual acuity after vitrectomy. We investigated the potential use of transthyretin (TTR), a carrier of the retinol/retinol-binding protein, as a biochemical marker protein.

Methods We measured TTR using immunonephelometry in a group of patients (n=77) in longstanding (> 1 week) retinal detachment (n=29), fresh (< 1 week) retinal detachment (n=17), macular holes (n=20), or diabetic retinopathy (n=11). Vitreous samples were taken at the start of every vitrectomy procedure. For reference values, cadaver specimens (n=73) were used.

Results Reference values for vitreous TTR (median 18 mg/l; IQR 4-24 mg/l) comprised 2.2% of reference values for vitreous protein levels (median 538 mg/l; IQR 269-987 mg/l). Vitreous TTR values of patients were comparable in all disorders. Vitreous TTR values were higher in phakic (median 22.5 mg/l; IQR 10-27 mg/l) than in pseudophakic patients (median 12 mg/l; IQR 8-19 mg/l) (p=0.06). Postoperative change in visual acuity correlated well with vitreous TTR values found peroperatively (rs=0.408; p=0.012). Both change in visual acuity and lens status were the only variables which proved to explain the variance of TTR (multiple correlation coefficient: 0.494; phakic status: t=2.767; p=0.0084; and change in visual acuity t=2.924; p=0.0056).

Conclusion Vitreous fluid concentrations of TTR can be regarded as a biochemical marker for retinal function.

■ 3221

Target oriented selection of electrophysiological endpoints in preclinical and clinical trials

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Purpose The design of pre-clinical and clinical studies, especially multi-centre studies shall be discussed concerning the assessment of retinal function, retinal diseases and retinal circuitry as well as biochemistry and the selection of the proper electrophysiological "markers" for appropriate endpoints on the basis of knowledge on possible actions of particular compounds on visual function.

Methods Quality has to be ensured by extended standard operating procedures and case report forms that implement also data that test the quality of procedures and calibrations. This includes harmonized quality standards, quality control, central reading and monitoring as well as data management.

Results On the basis of standards and recommendations of International Society for Clinical Electrophysiology of Vision and EVI.CT.SE, extended protocols have been developed, targeted at rod and cone function, including sensitivity assessment of the retina by V-log I-functions, submicrovolt recording for clinical trials in patients with very small responses, ON and the OFF pathway differentiation. Additionally, ERG recovery after a strong bleach is recommended to target alterations in the visual cycle that involves photoreceptors as well as RPE cells.

Conclusion First investigations with such refined standardized protocols have already been performed successfully showing a clear improvement of quality. Normative data are available for the various protocols.

■ 3223

The mysterious a-wave

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Purpose The electroretinogram (ERG) waveform usually consists of two major components, the a- and b-waves. Exceptions to the above rule are scotopic ERGs evoked to dim flashes, where only b-waves are recorded. The aim of this study was to examine if the no a-wave ERG was a feature of scotopic ERG only and try to explain why it occurs.

Methods Photopic (background: 30 cd.s.m⁻²; flash: -1.81 to 2.84 log cd.s.m⁻² in 0.2 log unit steps) and scotopic (-5.01 to -0.96 log cd.s.m⁻² in 0.4 log unit steps) ERG luminance-response functions were obtained from normal human subjects (n=30) and Long-Evans (n=8) and Sprague-Dawley (n=8) rats (scotopic ERGs only).

Results Human photopic ERG waveform always included an a-wave. With flash luminance, its peak time first increased, reached a maximal value and then decreased progressively and could be fitted to a fourth order polynomial function. In contrast, scotopic a-waves appeared only in ERGs evoked to flashes equal to or brighter than -3.01 log cd.s.m⁻². With increasing intensity, the peak time of the a-wave shortened following a linear model. On average, for each log-unit of flash attenuation, the scotopic a-wave shortened by more than 10 ms compared to 1.4 ms for the photopic one. Of interest, in rat scotopic ERGs evoked to the dimmest intensities the a-wave appeared to be replaced by a group of short latency oscillations that were best seen in recordings where the low frequency cut-off had been raised to 100 Hz.

Conclusion The a-b-wave rule seems to apply only to photopic ERGs where an a-wave was still identifiable in ERGs smaller than 1% of maximal amplitude. One wonders if the submicrovolt oscillations occurring prior to the onset of the b-wave of rat scotopic ERGs evoked to dim flashes could represent remnant of the original a-wave. Funded by CIHR

■ 3222

Separating physiological from pathological effects of drugs tested for retinal toxicity in animal models

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Purpose Drugs tested for retinal toxicity in animal models frequently induce physiological effects with or without pathological effects. The physiological effects may be interpreted to reflect toxic action. Here, different experimental approaches and modes of data analysis are described to separate physiological from pathological effects of drugs on retinal function and structure in laboratory animals.

Methods The flash electroretinogram (ERG) and the visual evoked potential (VEP) were used to assess respectively the functional integrity of the distal and proximal parts of the retina following intravitreal administration of drugs. Histology at the light microscopic level and immunocytochemistry were used to evaluate structural damage and to localize the site/s of action of the drugs.

Results The ERG responses and the VEP were recorded at different time intervals after intravitreal injection of different drugs in order to assess short-term and long-term retinal effects. In some cases severe functional deficit was identified, but it was transient in nature, and partial or even complete recovery was observed during a follow-up period of at least 4 weeks. Different modes of data analysis were applied in order to separate physiological effects from pathological ones.

Conclusion Drugs, used in the ophthalmic clinic, can be retinotoxic and induce permanent retinal damage, or may exert transient changes that can reflect drug interference with the normal physiological function of the retina. Data are analyzed to separate these possibilities in order to allow accurate assessment of drug toxicity. Furthermore, drugs exerting transient physiological effects can be used to study the normal mechanisms of visual information processing in the retina.

■ 3224

Electrophysiological assessment of optic neuropathies in neuro-ophthalmological practice

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Purpose Clinical challenge in diagnosis of optic neuropathies are atypical cases of either unknown visual loss or clinical cases in which the cause is identified, however, assessment of the visual function by psychophysical and morphological methods is not sufficiently accurate to assess the damage caused by lesion, or when functional follow-up is needed to assess the course. Such cases include inflammatory, compressive, paraneoplastic, infiltrative, toxic/nutritive or atypical demyelinating optic neuropathies.

Methods Electrophysiological methods will be interpreted with clinical history and presentation of clinical cases. Psychophysical methods and morphological presentation will be discussed in the context of why electrophysiology was needed either to clarify the diagnosis or to assess the functional status of the visual system.

Results Electrophysiological methods were informative in either dissociating the retinal involvement from that of optic nerve and to assess the functional preservation of the visual pathway. It is of importance that mfERG and pattern ERG can add to the diagnosis on macular disfunction where visual evoked potentials alone would be diagnostic for optic neuropathy.

Conclusion In neuroophthalmology, electrophysiological testing has its value especially in atypical cases and in situations where additional characterisation or functional assessment is needed. It is of importance to be able to dissociate functional characteristics of different components of visual pathway to assess the correct diagnosis and to assess whether the optic nerve may be endangered by progressive process, not readily discernible by other methods.

■ 3225

Novel mutations and electrophysiological findings in RGS9 and R9AP associated retinal dysfunction (Bradyopsia)

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Purpose To describe the phenotypic characteristics of patients with mutation in RGS9 or R9AP, which give rise to the disorder "bradyopsia"

Methods Patients were ascertained according to history, examination and electrophysiological recordings. ERGs were performed in excess of the ISCEV standard better to characterise retinal function, and included S-cone ERGs, ON-OFF ERGs, and dark adapted ERGs to a bright flash with extended inter-stimulus intervals. Blood samples were taken for DNA extraction from affected subjects and where possible unaffected relatives. Mutation screening of RGS9 and R9AP was performed.

Results Four subjects were found to harbour mutations in RGS9 or R9AP, with three being novel. Three subjects, two Pakistani sisters and an Afghani female, had mutations in R9AP. The remaining patient, a British male, had a compound heterozygous mutation in RGS9. All mutation positive subjects had characteristic electrophysiological findings with, in particular, severely abnormal photopic cone ERGs (with relative S-cone sparing) but normal dark adapted cone ERGs, and the need to use a prolonged inter-stimulus interval to allow recovery from a dark-adapted bright flash. Clinically, the patients had a stationary cone dysfunction syndrome, including mild photophobia; normal colour vision; normal fundi; and no nystagmus.

Conclusion Novel electrophysiological features are described in association with RGS9 and R9AP mutations. The distinctive electrophysiological features associated with RGS9 and R9AP mutations enable directed genetic screening, and allow the distinction from patients with Oligocone Trichromacy, who present with a similar clinical phenotype.

■ 3231

Ultrasound biomicroscopy for the analysis of the anterior segment

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The conventional ultrasound imaging allows to study easily the structures of the eye, even if the obtained informations have a maximal definition of 500-600 µm and a maximal depth of 30-40 mm. This definition, sometimes, is not enough to study the microscopical structures, such as cornea, iris, anterior chamber angle, ciliary bodies and zonula. The ultrasound biomicroscopy (UBM) was born to achieve a greater definition of the acquired images. This method, originally developed by Pavlin, Sherar, and Foster, allow the exploration of smaller and superficial structures, in particular of the anterior segment, with a spatial definition in vivo similar to histological preparations. The ultrasound biomicroscopy, thanks to high frequency of 50-100 MHz, can reach a depth of 5 mm with a definition of 50-60 µm, unthinkable with conventional ultrasound. The application fields of this technique are pathologies of conjunctiva, cornea, iris, zonula, ciliary body and lens. The best informations are given especially when we have to study the angle of anterior chamber and ciliary body in glaucoma, cancers of the anterior segment, the artificially outflow roads in glaucoma surgery and the accommodation mechanism. This technique have two main concerns, the repeatability of the measures is operator-dependent, as each ultrasound technology, and then it is a contact method. Even if new no contact and operator independent technologies are emerging, like interferometry and Scheimpflug Camera, UBM is actually undoubtedly the gold standard for the study of the anterior segment structures.

■ 3233

In vivo confocal microscopy of the cornea and conjunctiva

BAUDOUIN C

Boulogne

ABSTRACT NOT PROVIDED

■ 3232

Anterior segment OCT in corneal diseases and surgery

NUBILE M

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Purpose Optical coherence tomography (OCT) after extended use in diagnosis of retinal diseases has now become a new cross-sectional imaging approach useful for anterior segment (AS) imaging. The potential advantages and limits using AS-OCT in corneal diseases and surgery are presented.

Methods Systematic literature review search and clinical examples focused in the fields of corneal ulcerations, opacities and corneal graft penetrating and lamellar surgery.

Results AS-OCT technology, using 1310 nm wavelength, offers some advantages as compared to high frequency ultrasound system, the main being the absence of contact with the eye. It provides relatively accurate morphology and measurements of different structures of the AS and of the cornea. Corneal loss of transparency in general permits visualization of the deeper AS structures facilitating surgical choice in complex cases. In contrast ciliary bodies are barely visualized, in contrast to UBM, due to light absorption by pigmented iris layers. Morphometry of corneal structures is particularly useful in the preoperative evaluation of thinning disorders, corneal ulcerations, loss of transparency and after penetrating and lamellar surgery, including assessment of LASIK flaps and of deep anterior keratoplasty interfaces and endothelial keratoplasty lenticules adhesion.

Conclusion AS-OCT presents advantages in the clinical practice in the field of corneal disease and surgery. This technology alone should not substitute a comprehensive clinical examination but may offer valuable insights in the quantification and observation of fine details which can be obscured or not detectable during conventional slit-lamp examination.

■ 3234

Diffuse keratoconjunctival diseases: an in vivo confocal microscopic study

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Purpose To establish in vivo confocal microscopic features of diffuse ocular surface disease.

Methods IVCM of diffuse OSD such as chemical burns, inflammatory disease related limbal stem cell deficiency, conjunctival intraepithelial neoplasia and OS infections was carried out using the Rostock Cornea Module RCM and HRTII. In some patients subsequent biopsy or surgery allowed for histological correlation of the observed IVCM features.

Results Multiple clinical and histological correlations have enabled us to establish diagnostic features of dendritic cells, inflammatory cells, metaplastic and dysplastic superficial and basal epithelial cells, goblet cells, intraepithelial cysts, corneal new vessels, subtypes of acanthamoeba cysts and other nonspecific features. These features can be seen individually or in combination in different diseases entities.

Conclusion IVCM can help in making rapid clinical diagnoses without resort to biopsy or impression cytology. This in turn allows for early initiation of appropriate treatment.

Commercial interest

■ 3235

Anterior segment OCT versus UBM in the surgical management of pseudophakic bullous keratopathy

GICQUEL JJ

Poitiers

Anterior Segment-OCT (AS-OCT) is a non contact high Resolution Imaging system. It uses 1310 nm Coherent light and can scan up to 6 mm in Depth and thanks to a maximum scan width of 16 mm it can provide limbus to limbus cross sectional images even thru an opaque cornea. The acquisition is fast up to 8 Frames per second (almost video like). We decided to compare it to the Ultrasound Biomicroscopy (UBM), that is considered to be the gold standard of AS imaging. In a randomized comparative case series in patients with pseudophakic bullous keratopathy (PBK), we studied with AS-OCT and UBM the anatomical modifications after a triple procedure involving: formerly implanted AC IOL removal followed by the fixation of a Verisyse Aphakic IOL either over or under the Iris. The AS anatomy was best respected in patients who had the Verisyze IOL implanted behind the iris. Measurements obtained with AS-OCT and UBM were well correlated. AS-OCT is a reliable and easy to use option for accurate AS imaging before and after surgery in patients with PBK.

■ 3236

Anterior segment OCT in glaucoma

CIANCAGLINI M

ABSTRACT NOT PROVIDED

■ 3241

The different domains of the point spread function - Small angle vs large angle

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Purpose The Point-Spread-Function peaks at 107, and drops off to 10-2 at 90 degrees, a dynamic range of 109. Important changes in the PSF already occur within the first 1 minute of arc, so spatially the dynamic range is 105. Very different experimental techniques are needed to describe the small angle (aberrometry, double pass) and the large angle (straylight) domains. What optical irregularities achieve such PSF?

Methods Literature models for the aberration structure of the human eye media, in particular Thibos et al. JOSA A 2002 were used to derive the PSF. The Thibos et al. model was extrapolated to allow inclusion of the high orders of aberration not normally included in aberrometry, but potentially visible with double pass, so as to encompass as much as possible from the PSF.

Results The modeled aberration structures of the eye covers the central part of the PSF up till about 0.3 degrees if extreme high orders were included. For angles >1 degree predicted values were far below the actual values of the PSF. On the other hand, double pass techniques grossly overestimate PSF values at angles of 0.3 degree and above. Using an alternative model for the aberration structure of the eye the PSF could be described up till 1 degree. Small particle scattering was essential to predict the PSF for angles above 1 degree.

Conclusion In the assessment of disturbances to the optical media two domains must be discriminated: the aberration domain and the small particle domain, with corresponding parts (small angle vs large angle) to the PSF. Straylight originates from irregularities of small characteristic size (10 micrometer and below), as opposed to disturbances to the central part of the psf (<0.3 degrees), originating from large scale irregularities.

Commercial interest

■ 3243

Optical simulation of large and small angle scatterers

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Purpose However corneal scattering is clinically often quantified as haze (backscattered light), the visual function is affected by the forward light scattering in the anterior ocular media. The angular light distribution of the scattered light is inherent to the interaction between the light and the scattering media. The purpose of this study is to describe an optical model of the scattering tissues in the cornea and compare its light propagation to experimental single pass scattering measurements.

Methods Histologic data and confocal imaging of the cornea revealed that a significant portion of corneal scattering originates by keratocytes and scar tissue created during a wound healing process, e.g. after refractive surgery. Keratocytes were modeled as small spheres, angular scattering distribution is derived on the Mie theory. The parameters were optimized to meet the light distribution measure by a single pass experiment through excised rabbit corneas.

Results Single pass scatter measurements of excised rabbit corneas revealed a narrowly forward peaked scattering distribution (FWHM 30 arcmin), corresponding to a distribution of scattering spheres with $36\mu\text{m} \pm 15$ radius and a relative refractive index of 1.0024. Scattering ratio (SR), equal to the scattering cross section, varied from 0.2 (clear) to 0.7 (scarred). The SR was correlated with the scar tissue thickness (0.79) and haze grade (0.51), Pearson's coefficient.

Conclusion Despite the non-spherical geometry of keratocytes, we presented a simple model to describe the corneal scattering distribution, controlled by physically relevant parameters as observed in histologic and confocal microscopy. Optical simulations based on light propagation through random phase maps may result in more realistic scattering models.

■ 3242

Challenges in optical measurement of scatter

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Purpose Measurement of scattered light in biological media and particularly the optical media of the human eye is associated with two main challenges: The extremely low intensity of the scattered light and artefacts associated to tissue handling and preparation.

Methods Our strategies in reducing the errors associated with the above mentioned challenges include the utilisation of properly designed illumination schemes and appropriate procedures and devices for excising and mounting the specimens.

Results Appropriate techniques that incorporate precise knowledge of the optical PSF of the system may prove useful for the accurate measurement of scatter light. Diffraction through the utilisation of narrow (e.g. 0.5mm) diaphragms may prove useful for the broadening of the optical PSF (while preserving knowledge of its shape) and therefore allow optical measurements of a wider range of angles.

Conclusion Optical measurements of scatter may prove useful not only for the macroscopic characterisation of the optical quality of the optical media of the eye but also for the extraction of information concerning the microscopic structure of the irregularities causing the loss of transparency.

■ 3244

Straylight in function of wavelength and refraction

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Purpose To determine dependencies of straylight on ocular biometry.

Methods This prospective study includes 518 eyes of 277 volunteers with healthy eyes of various iris colors and ethnicities who had their retinal straylight tested using the psychophysical Oculus C-Quant device. Ocular axial length and refraction were also measured, respectively using the Zeiss IOL master and the Nidek ARK-700 autorefractometer.

Results The measured retinal straylight was validated by comparing our data with the age model described in the literature as $\log(s) = P1 + \log(1 + (\text{Age}/65)^4)$. Our data was found to agree well, albeit that P1 was slightly higher (0.931 versus 0.87). Subtracting this model from the measured straylight values, a quadratic increase was found in function of axial length L. A similar model was defined for the spherical equivalent refraction SE and retinal image size I. This corresponds to an increasing straylight for increasing degrees of myopia. No correlation with keratometry or corneal astigmatism was found.

Conclusion Retinal straylight increases not only with age, but also with axial length. Further study is needed to identify the cause of this dependency.

■ 3245

Intraocular straylight screening in medical testing centres for driver licence holders in Spain

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Purpose To test the performance of the C-quant straylight meter during the daily routine work in medical testing centers for driver license applicants and driver license holders in Spain.

Methods Altogether 914 subjects, of which 376 younger than 35 years, 428 between 35 and 60 years and 110 over 60 years were measured with the C-quant in three medical testing centres (Barcelona, Zaragoza and Palma de Mallorca) in 2006. Technicians were instructed once and the measurements were done during the daily routine work. We recorded: age, BCVA, self-reported subjective blinding at night; and from the C-quant: straylight parameter (log s), measurement quality parameters (ESD, Q) and test duration.

Results Total C-quant test duration increases slightly with age from a mean of 7 min (<35 years) to a mean of 9 min (>60). At first attempt, 82% of all subjects produced reliable results (ESD < 0.12). The straylight parameter for this group was independent of ESD and ESD was independent of total test duration. The known age dependence of the straylight parameter and the weak correlation with BCVA was confirmed. The distribution of subjective blinding at night was very different between test centres. Subjects with "very strong" subjective blinding had significantly higher straylight values than subjects with "no" subjective blinding. Subjects avoiding night driving had significant higher straylight values than subjects driving at night.

Conclusion The C-quant measure is reasonable fast. Good subject instruction is important to get first attempt reliable results. Self-reported subjective blinding results depend strongly on the interviewer. The C-quant is an improvement over present methods, because it is objective and cannot be influenced.

■ 3246

Quality of life in cataract patients and the role of stray light

ASPINALL P (1, 2, 3)

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(2) Adrian Hill, Heriot Watt University

(3) Heriot Watt University, Rod Graves

Purpose The aim of this study carried out at the Princess Alexandra Eye Pavilion in Edinburgh was to explore the similarities and differences in criteria for cataract surgery in current practice across 5 consultant teams at the hospital. In addition, a number of specific research questions were addressed. Among these, and the basis of this presentation, is the implication of using quality of life as a key referral criterion; and the relations between pre-operative measures of stray light and quality of life indicators.

Methods 105 patients attending the cataract clinics at the hospital participated in the study. In addition to normal clinical practice, data was gathered on visual function (Snellen, LogMar, Pelli Robson contrast sensitivity); other clinical measures (Oxford cataract grading system and C-quant); and quality of life indicators (VF-14 and Vsq questionnaires).

Results Results will be presented in two parts. Firstly showing how the quality of life and the C-quant measures relate to current practice. Secondly exploring inter-relationships between the C-quant and individual and aggregate quality of life scales as a basis for alternative referral criteria.

Conclusion General quality of life measures are loosely related to current referral practice for cataract surgery. On the other hand stray light (C-quant) measures do seem more closely related to current practice

■ 3251

Women in ophthalmology: From bench to boardroom

GRAVES A

President and CEO, Santen Incorporated

Dr. Adrienne Graves began her career as a visual scientist, with stops at Brown University, Harvard Medical School, University of Michigan, and University of Paris. Her passion for studying the visual system and the pathophysiology of disease processes led her to the pharmaceutical industry. After positions in R&D at Alcon, she is now President/CEO of Santen's US operations. Dr. Graves credits her time in Paris for expanding her horizons in many ways, including an appreciation for cultural diversity. This is especially important in her role as a Corporate Officer for Santen, a global ophthalmic pharmaceutical company based in Japan.

■ 3261

Oncocytic lesions of the ophthalmic region

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Purpose To make a nationwide clinicopathologic study of oncocytic lesions in the ophthalmic region and to characterize their cytokeratin(CK) expression.**Methods** All histologically diagnosed oncocytic lesions in the ophthalmic region registered in Denmark over a 25-year period were collected and re-evaluated using a monoclonal antimitochochondrial antibody (MU213-UC). Clinical data were registered. Immunohistochemical characterization was performed with a panel of anticytokeratin antibodies.**Results** A total of 34 oncocytic lesions were identified. The incidence that required surgical intervention in the Danish population could be approximated to 0.3 lesions per million capita per year. The age of the patients ranged from 45 to 89 years with a peak incidence in the 8th decade. Female gender was twice as common as male. Lesions were typically described as red-brown, cystic and slow growing. The antimitochochondrial antibody MU213-UC produced a distinct and intense immunostaining. 26 of the lesions originated from the caruncle, three in the conjunctiva, two in the lacrimal sac, one at the semilunar plica, one on the eyelid margin and one peripunctal. Lesions were histologically classified as adenoma(oncocytoma) (26), hyperplasia (4) and metaplasia (4). Basal-type oncocytic cells reacted with antibodies against CK5/6, CK7, CK8, CK13, CK14, CK17, CK18, CK19 and suprabasal cells with CK4, CK7, CK8, CK18 and CK19. Antibodies against CK1+10 and CK20 showed no reaction.**Conclusion** Oncocytic lesions of the ophthalmic region most frequently present as a caruncular oncocytoma. The cytokeratin profile is similar to the lacrimal - and accessory lacrimal gland duct elements and supports the theory that these lesions originate from lacrimal - and accessory lacrimal glands.

■ 3263

Heterogeneity in uveal melanoma assessed by multiplex ligation-dependent probe amplification (MLPA)

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Purpose To study intratumour heterogeneity in primary uveal melanoma (UM) by MLPA in microdissected formalin-fixed, paraffin-embedded (FFPE) tissues.**Methods** DNA was extracted from 2-9 areas microdissected from 32 FFPE UMs. Thirty-one loci on chromosomes 1p, 3, 6 and 8 were tested for copy number changes using the SALSA MLPA P027.B1 assay (MRC Holland). MLPA data were displayed as dosage quotients (DQs), which were classified to 5 ranges (0.35-0.64 deleted; 0.65-0.84 equivocal deletion; 0.85-1.14 normal; 1.15-1.35 equivocal amplification; >1.35 amplified). The tumour was considered heterogeneous at a locus, if a) the difference in DQs of any two areas was higher than 0.2 (value determined by ROC analysis), and b) the DQs of the areas belonged to different ranges.**Results** Genetic abnormalities were detected in all 32 UMs. Monosomy 3, the most significant metastasis predictor, and gain of 8q genes MYC or DDEF1 were detected in at least 1 microdissected area of 22 (69%) and 28 (87%) of the tumours, respectively. The comparison of MLPA data obtained from different areas of UMs showed heterogeneity in 1-24 loci across chromosomes 1p, 3, 6 and 8 in 26 (81%) tumours. Interestingly, trisomy 3 was observed in 3 (9%) UMs and these tumours showed the highest degree of heterogeneity (>23 heterogeneous loci). Intratumour heterogeneity of 3p12.2 (ROBO1) and 6p21.2 (CDKN1A) were most common and present in more than 35% of the tumours.**Conclusion** Heterogeneity of chromosomal abnormalities of 1p, 3, 6 and 8 is present in many UM. Taking one random tumour sample for prognostic testing, therefore, may not be representative of the whole tumour.

■ 3262

A Western blot analysis of P-glycoprotein in retinoblastoma

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Purpose Literature regarding role of P-glycoprotein (P-gp), a multi-drug resistance (MDR) protein, in retinoblastoma (Rb) is scanty and research has mainly concentrated upon immunohistochemistry. We analyzed P-gp expression in Rb by Western blotting (WB) and correlated with histopathology (HP).**Methods** Prospective analysis of P-gp was done between May 2008-May 2009 on 15 human Rb tissue specimens after enucleation, either as primary treatment [Group I;n=10] or secondary to chemotherapy (vincristine, etoposide and carboplatin) [Group II;n=5]. Samples collected immediately after enucleation in RPMI-1640 supplemented with 10% fetal bovine serum, were subjected to WB. HP details (routine H & E) were considered for tumour differentiation and invasion of optic nerve/ocular coats. P-gp expression was graded semi-quantitatively as negative, low or high.**Results** By WB, P-gp expression was found in 30% of patients in group I (3/10) and 80% of patients in group II (4/5). All expressions in group II were high expression. On HP, in Group I: 70% poorly differentiated (PD) and 30% well differentiated (WD) tumours; 5/10 had involvement of optic nerve/ocular coats/others. In Group II: 60% PD and 40% WD tumors. 1/5 had involvement of optic nerve/ocular coats. 2/5 chemo-treated eyes were phthisical.**Conclusion** By WB analysis, P-gp was expressed more frequently in Rbs treated by chemotherapy. No significant relation between tumor differentiation, tumor invasion and P-gp expression was found. Though this may be the first study of its kind with promising results, similar studies could be done in more patients with further parameters, especially while managing chemoresistant cases of Rb in the only remaining eye of a child.

■ 3264

Revisiting the value of assessing the mitotic rate of choroidal melanoma

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Purpose To re-evaluate mitotic rate (MR) as an indicator of malignancy grade in uveal melanoma (UM) and as a predictor for UM-related mortality.**Methods** UM patients treated 1993-2006 by local resection or enucleation were included. Data on largest basal diameter (LBD), ciliary body involvement, extraocular extension, cell type, closed loops, MR per 40/HPF, cytogenetics were collected. Mortality data were obtained from NHS Cancer Registry.**Results** 918 patients (520 M; 398 F) had median age of 64.6 yrs with a median follow-up of 3.46 yrs (range 0.02-13). The UM had mean diameter of 14.9 mm with ciliary body involvement in 43.0%, epithelioid cells in 63.3%, closed loops in 41.2%, extraocular spread in 11.3%. Cytogenetics in 337 patients showed disomy 3 and 8 in 27%, monosomy 3 in 10.7%, chromosome 8 gains in 24.0%, both these abnormalities in 38.3%. The median MR was 3(range 0-61, SD 6). High MR correlated with ciliary body involvement (p = 0.001), epithelioid cells(p = 0.009), closed loops(p<0.0001), extraocular spread(p=0.027) & monosomy 3(p<0.0001;Mann-Whitney). MR also correlated with LBD(p=0.0001; t-test). Metastatic death occurred in 243 patients (26.7%). Kaplan-Meier analysis showed MR >4/40 HPF to be associated with increased 10-year metastatic mortality from 32.5 (95% confidence intervals [CI] 25.7-39.3) to 65.5 (95% CI 55.7-75.4; log rank statistic 53.28, p<0.0001). Cox multivariate analysis showed MR to be an independent predictive factor for metastatic death (p<0.0001). MR was predictive of metastatic death also in patients without detectable monosomy 3 (Log rank statistic 10.38, p = 0.002).**Conclusion** MR correlates with other known risk factors for metastatic death and independently predicts mortality even when cytogenetics show disomy 3.

■ 3265

Granular cell tumour of the lacrimal gland

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Purpose To report the clinical and histopathological characteristics of a patient with a granular cell tumour (GCT) of the lacrimal gland.

Methods Surgical excision and histological examination.

Results A 38-year old male presented with a painful swelling located temporally in the right upper eyelid. Clinical examination revealed proptosis and displacement of the right eye and a tumour was palpated at the site of the lacrimal gland. MRI scan revealed a solid tumour in the lacrimal fossa. The tumour was excised. Microscopically the tumour was composed of tumour cells with coarsely granular cytoplasm. The tumour cells were arranged in clusters and ribbons separated by collagen bundles and no necrosis or mitosis were present. The granules were PAS positive, diastase resistant and the tumour cells expressed focal staining for S100. Electron microscopy showed numerous secondary lysosomes. The diagnosis is consistent with a GCT.

Conclusion This case presents for the first time a GCT of the lacrimal gland.

■ 3267 / 349

Ophthalmologic outcome of 40 spheno-orbital meningioma resections: 15 years long-term results

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Purpose To evaluate the operative results of spheno-orbital meningiomas regarding resectability, recurrence and ophthalmological outcome.

Methods Retrospective study of 40 patients who underwent surgical resection of spheno-orbital meningiomas followed by bony reconstruction between 1992 and 2008.

Results Mean age of 50 years and sex ratio of 37 women and 3 men. The most common preoperative sign was proptosis (90%), visual acuity (VA) and visual field deficits (55%). Periorbital tumor infiltration is a predictive factor of decreased VA in case of initial VA deficit. Optic canal invasion and resection don't influence VA recovery. Postoperatively, 10 patients showed severe permanent visual field deficits, versus 17 patients showed no visual field deficits. After surgery, proptosis improved for all patients with a preoperative mean proptosis of 5.6 +/- 3.6 mm versus 2.2 +/- 2.7 mm postoperatively (p<0.001). Lateral orbital wall resection and bony reconstruction are the two major factors which are efficient in reducing proptosis. Mean follow-up period was 7 years. Clinical tumor recurrence was observed in 10 patients (27%), radiological tumor recurrence without clinical signs was observed in 7 patients. 4 patients underwent re-operation and 3 patients were treated by radiation. Resection quality is the only predictive factor of a clinical recurrence.

Conclusion Periorbital tumor infiltration is a predictive factor of non-improvement of visual deficits (VA or visual field deficits). Peri-orbital resection leads to a better esthetic result with a better proptosis reduction. Complete and subtotal (> 90%) tumor resection gives a long-term survival free of clinical recurrence for 80% of our patients.

■ 3266 / 348

Immunohistochemical findings and proliferation markers in uveal melanomas after gamma-knife radiosurgery

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Purpose We describe immunohistochemical characteristics and tumor cell proliferation in 12 eyes with uveal melanoma enucleated after Gamma-Knife radiosurgery (GKR) with different treatment doses.

Methods Tumors were stained for the specific melanocytic markers S-100, HMB-45 and Melan A. The immunoreactive score (IRS, Remmele-score) was calculated from percentage of positive cells and staining intensity. Proliferating cells were identified with PC-10 and MIB-1 staining and counted. All markers were evaluated at the tumor apex, center, base and margins. Results were compared between eyes enucleated for tumor recurrence or treatment related side effects. Influence of treatment dose and time to enucleation were analyzed.

Results Only 1 of 12 melanomas stained positive for S-100 (median IRS 0). HMB-45 and Melan A were both positive in 11 of 12 cases (median IRS 9 and 10.5, respectively). Staining intensity was higher at the tumor apex, base and margins than in the tumor center, which frequently showed necrosis. PC-10 and MIB-1 showed proliferating cells in all melanomas, without relation to tumor recurrence (n=5), radiation dose (25-50 Gy) or time between GKR and enucleation (1-47 months). We found markedly more proliferating cells at the tumor margins.

Conclusion Proliferating cells were found even in cases with clinically stable disease. The finding of cycling cells at the tumor margins and necrosis in the tumor center might reflect the inhomogenous dose distribution used for GKR, with the tumor center receiving almost twice the dose of the surface and margins. It might also be an indicator for an intrinsically higher proliferation rate at the margins of the melanoma.

■ 3311

3 Intravitreal ranibizumab injections series for the treatment of neovascular age-related macular degeneration (AMD), 18 months follow-up

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Purpose To evaluate the functional, anatomical, vascular flow effects of intravitreal Ranibizumab injections (IVT) for retrofoveal neovascular AMD, done with a series of 3 IVT protocol, and the recurrences frequency at 1 ½ year evolution.

Methods 227 eyes of 179 patients, 58 men, 121 women, with retrofoveal neovascularisation complicating AMD. Patients received intravitreal Ranibizumab, 3 times, every 4 weeks in an inductive treatment. The next injections depended on the follow-up results, and were done by series of 3. First and 2 months' interval follow-up exam included ETDRS visual acuity (VA), complete ophthalmic examination, fluorescein and indocyanine (ICG) angiography, and optical coherence tomography (OCT). VA and OCT were done before each IVT. We want to evaluate the incidence of this protocol on the frequency of recurrences so on the number of IVT needed.

Results VA improved in 48% cases, more than 10 letters in 25% cases. Angiographic leakage reduced about 70% in 53% cases. At ICG, vascular flow, vessel's diameter were 2/3 time less in 62% cases. Diffuse edema was 30% reduced in 85%, pigment epithelial detachment was flattened and less dense in all cases, by OCT. Most of patients had good functional, anatomical, vascular flow, results, with less IVT needed. Inductive treatment was sufficient in 113 cases, more series were necessary in 114 cases. Mean stability period was 3 months.

Conclusion The results, with generally improved visual function, less and/or stable neovascular activity in FA-ICG-OCT, less recurrences, suggest series of 3 Ranibizumab IVT seem effective, less restrictive, more retinal protective, lastly cheaper. This protocol seems attractive, its indication must be optimized.

■ 3313

Combined intravitreal bevacizumab and triamcinolone for exudative age-related macular degeneration

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Purpose To report on the combined application of intravitreal bevacizumab with triamcinolone acetonide for treatment of exudative age-related macular degeneration.

Methods The clinical interventional case-series study included 16 patients (16 eyes) with exudative age-related macular degeneration who had previously received 3.5±1.8 mono-injections of bevacizumab (1.5 mg) without significant improvement in visual acuity or reduction in macular exudation. All patients underwent a combined intravitreal injection of bevacizumab (1.5 mg) and triamcinolone acetonide (about 20 mg). Main outcome measures were visual acuity and macular thickness as determined by optical coherence tomography. All patients were re-examined at 2 to 3 months after the intervention.

Results Visual acuity improved significantly ($P=0.03$) from 0.80 ± 0.40 logMAR prior to the combined injection to 0.65 ± 0.42 logMAR at 3 months after the injection. An improvement by at least one Snellen line was found for 8 subjects, an increase by at least 2 lines for 5 subjects, and an improvement of 3 or more lines for 2 subjects. One patient lost one line, and one patient lost 3 lines. The central retinal thickness decreased significantly from 272 ± 62 to 220 ± 47 micrometer ($P=0.03$). At the 6 months follow-up examination, the central retinal thickness increased again to 319 ± 142 micrometer, which was not significantly ($P=0.30$) different from the baseline measurements.

Conclusion The combined intravitreal application of bevacizumab and triamcinolone may temporarily be helpful in the treatment of exudative age-related macular degeneration if previous intravitreal bevacizumab mono-injections failed to improve vision and reduce macular edema

■ 3312

Outcome of exudative aged-related macular degeneration (ARMD) after 3 intravitreal injections of bevacizumabCREUZOT C, BIDOT ML, SOUCHIER M, MALVITTE L, BRON AM
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Purpose To evaluate the efficacy of intravitreal injections of bevacizumab in exudative ARMD.

Methods Retrospective study including naive patients suffering from exudative ARMD whatever the type of neovascularisation. All the participants were treated with three monthly 1.25 mg intravitreal injections of bevacizumab. The primary objective was far and near visual acuity (VA) 1, 3 and 6 months after the third injection. The secondary objective was the residual activity of neovascularisation assessed with fluorescein and ICG angiography and retinal thickness evaluated with OCT3.

Results 71 eyes of 66 patients were included. Neovascularisation was occult, visible or combined in 65%, 20% and 15% of the cases, respectively. A statistically significant improvement between pre and post-injection VA (LogMAR) was observed one month after the third injection, 0.88 ± 0.57 and 0.77 ± 0.60 , respectively, $p=0.001$. An active neovascularisation was still present at that time in 57.7% of the eyes and 34% at 6 months needing further bevacizumab injections (3.85±0.96 per eye). VA was similar at 1, 3 and 6 months ($F_{1,2}=1.54$; $p=0.46$). A complication occurred in 3 eyes.

Conclusion Three bevacizumab intravitreal injections led to a significant VA improvement. However, more than half of the eyes had an active neovascularisation after these 3 injections.

■ 3314

Focal photodynamic therapy for idiopathic polypoidal choroidal vasculopathy

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Purpose To evaluate the focal treatment with photodynamic therapy (PDT) to treat idiopathic polypoidal choroidal vasculopathy (IPCV).

Methods Retrospective study of 8 eyes of 6 patients with visual loss and diagnosed of IPCV. Diagnosis was confirmed with indocyanine green (ICG) angiography. We treated with PDT only the "hot spots" or the maximum hyperfluorescence focal area. In the first month, funduscopic and visual acuity examination using ETDRS optotype were performed. In the third month all the examinations including a fluorescein and ICG angiography were repeated. Afterwards, controls had taken place every 6 months.

Results Five women and one men were included in the study. Its age was between 64 and 85 years. Mean follow-up was 18.1 months (range: 6-35 months). Mean initial and final visual acuity were 20/100 and 20/80 respectively. We observed a visual acuity improvement in 6 eyes and a visual stability in 2 eyes. Five eyes needed only one treatment, 2 eyes two PDT sessions and only one eye required 5 PDT sessions.

Conclusion In our study, focal photodynamic therapy of "hot spots" has shown to be an effective, non invasive and secure treatment for idiopathic polypoidal choroidal vasculopathy.

■ 3315

Prospective evaluation of macular and parapapillary morphology and their association with cognition in childhood and old age

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Purpose We investigated the potential association between cognitive function and retinal morphology based on shared age-related changes in a cohort of older people.

Methods Participants (n=250) from the Scottish Mental Survey 1947 undertook the Moray House Test (MHT) of general cognitive ability (IQ) at age 11 and 70. All were born in 1936. Fundus photographs were assessed by an ophthalmologist masked to the results of these tests.

Results Univariate analyses of variance were computed to compare the effects of retinal morphological features on cognitive test scores. Presence of retinal drusen was associated with lower age 70 IQ scores when compared to those without this abnormality. This difference approached statistical significance ($F(1,166)=3.77$, $p=0.054$, $\eta^2=0.22$). Scores on the same cognitive test administered at age 11 were significantly lower in those with retinal drusen when compared to IQ scores from a group without this abnormality. This difference was statistically significant ($F(1,158)=4.03$, $p=0.046$, $\eta^2=0.025$), though the effect size was small. But when age 11 IQ was included as a covariate in the earlier model, age 70 IQ scores were no longer significantly different by retinal drusen grouping ($F(1,155)=1.47$, $p=0.228$, $\eta^2=0.009$). Similar analyses using parapapillary atrophy yielded null findings.

Conclusion Initial analysis of the age 70 IQ data implied that macular drusen could serve as a potential bio-marker of cognitive decline. But the added information from the age 11 IQ data raises the possibility of reverse causation: rather than late-life retinal damage being a marker of cognitive decline, early-life cognitive ability might account for this retinal change in late-life.

■ 3317 / 426

Combined 23-gauge sutureless pars plana vitrectomy (ppV), injection of recombinant tissue plasminogen activator (rtPA), expansile gas and bevacizumab treating acute subretinal haemorrhage (SRH) in exudative AMD

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Purpose To evaluate the effect of 23-gauge core ppV with consecutive intravitreal injection of rtPA, gas and bevacizumab for the displacement of SRH in patients with exudative age-related macular degeneration (AMD).

Methods The retrospective, non-randomized case study included 15 eyes of 15 patients aged 74 to 89 with SRH resulting from exudative AMD. Each patient was treated with 23-gauge ppV and injection of 0.05 ml rtPA (50 µg), 0.3 ml of sulphur hexafluoride (SF6) gas and 0.05 ml bevacizumab (1.25 mg). Visual acuity (VA), lesion size measured by fluorescein angiography (FA) and retinal thickness as determined by optical coherence tomography (OCT) were evaluated pretreatment as well as 1, 3 and 6 months postoperatively.

Results The mean visual acuity pretreatment was 20/400. The figure remained constant until month 3 postoperatively. At 6 months, the mean VA had improved to 20/200. No patient had reading ability before treatment. 1 month postoperatively reading VA was achieved in 6%, rising to 27% at 6 months. There was a reduction in retinal thickness in 8 patients measured by OCT. FA showed a reduction of lesion size in 10 out of 15 patients.

Conclusion The combined application of rtPA, gas and bevacizumab via 23-gauge PPV for the treatment of SRH secondary to exudative AMD appears to be a safe and effective method. In our study, the combined treatment including the injection of bevacizumab enabled 27% of patients to achieve reading ability at 6 months postoperatively.

■ 3316 / 425

Retinal angiomatous proliferation: is intravitreal ranibizumab therapy enough?

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Purpose To report the use of Ranibizumab monotherapy versus Ranibizumab combined with additional photodynamic therapy (PDT) in the management of retinal angiomatous proliferation (RAP).

Methods The data from a non-randomised series of 25 patients with RAP lesions attending the Bristol Eye Hospital age-related macular degeneration (AMD) treatment clinic from April to October 2008 was analysed. The RAP lesions were classified according to Yannuzzi's system as stage 1, 2 or 3 based on clinical, ocular coherence tomogram and angiographic features. Two treatment regimes were identified (1) Lucentis monotherapy and (2) Lucentis monotherapy with additional photodynamic therapy (PDT). The main outcome measures were the % of eyes losing < 15 letters, % of eyes gaining ≥ 15 letters and the mean change in visual acuity.

Results Mean LogMAR visual acuity was 52.8 letters before treatment. Mean follow-up time is 9.5 months. Fourteen eyes had been treated with PDT before or after the use of Ranibizumab. Two eyes received PDT+IVT only (Stage 2 patients). 77% of eyes had an improvement in visual acuity. 18.5% of eyes have had improvement of 15 or more letters. Mean change in visual acuity was +7.2 letters. 57% and 60% of patients with stage 1 & 2 disease respectively, received PDT.

Conclusion Combined therapy is effective in improving visual acuity and reducing central retinal thickness (CRT) in all patients with stage 1 lesions and most of patients with stage 2 lesions. Combined therapy offered greater gain in visual acuity in all treated patients. Stage 1 and 2 patients benefit more from the treatment if the condition is recognised and treated early.

■ 3321

Findings in electrooculography and multifocal electroretinography in patients after bevacizumab treatment for age-related macular degeneration

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Purpose To assess usefulness of electrooculography (EOG) and multifocal electroretinography (mfERG) in monitoring electrophysiological changes in patients after bevacizumab treatment.

Methods 33 patients with age-related macular degeneration underwent ophthalmic examination including visual acuity, EOG, mfERG (Vision Monitor, Metrovision, France). Visual acuity test, sensory EOG protocol and mfERG with 61 scaled hexagons for low vision protocol were used during electrophysiological exams before and 1 month after each bevacizumab injection.

Results before bevacizumab injection mean visual acuity was $0,181 \pm 0,160$, mean Arden's coefficient was $199,692 \pm 54,785\%$, mean P1 wave amplitude < 2 deg was $29,062 \pm 14,89$ nV/deg, mean P1 wave < 2 deg implicit time was $47,015 \pm 12,832$ ms. 1 month after bevacizumab injection we observed no statistically significant mean visual acuity decrease ($0,181 \pm 0,160$ vs. $0,162 \pm 0,140$ $p=0,757$), no statistically significant mean P1 wave amplitude < 2 deg decrease ($29,062 \pm 14,89$ vs $25,077 \pm 17,996$ nV/deg $p=0,544$) and no statistically significant mean P1 wave < 2 deg implicit time decrease ($47,015 \pm 12,832$ vs. $46,115 \pm 10,313$ ms $p=0,845$). We observed positive correlation between visual acuity and P1 wave amplitude < 2 deg ($r=0,540$, $p<0,05$).

Conclusion Electrooculography and multifocal electroretinography are useful exams in monitoring electrophysiological changes in patients during bevacizumab treatment.

■ 3323

Extensive macular atrophy with pseudodrusen-like appearance (EMAP), a new clinical entity

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Purpose To describe a previously unreported clinical entity of progressive extensive macular atrophy and pseudo-drusen appearance in middle age patients.

Methods The database of the outpatient clinic for genetic sensory diseases was screened for patients over 40 years with uncharacterized macular dystrophy. Patients with extensive macular atrophy and pseudodrusen-like appearance were included. Clinical investigations included visual acuity, eye examination, color fundus imaging, OCT scan, autofluorescence, Farnsworth 15-HUE color testing, Goldman perimetry and ISCEV full-field ERG.

Results Eighteen patients of 45 screened records matched the inclusion criteria. Bilateral polycyclic well-delineated chorioretinal atrophy extending to the temporal vascular arcades, with a larger vertical axis and without sparing of the fovea featured the macular lesion. The pseudodrusen-like appearance was widespread throughout the posterior pole and the peripheral retina. In the extreme periphery, paving stone lesions were mostly located in the inferior quadrants. In contrast to age-related macular degeneration, a rapid progression of the atrophy was observed with an early involvement of the foveal zone, thus leading to a severe visual loss. All the patients except two were legally blind at the end of the follow-up. In all patients, the scotopic and photopic ERG responses were reduced.

Conclusion The cause of Extensive Macular Atrophy with Pseudodrusen (EMAP) remains unknown. Extensive Macular Atrophy with pseudodrusen (EMAP) should be considered as a possible pattern of severe macular dystrophy occurring in the middle-aged adult.

■ 3322

Importance of electron microscopy in neuronal ceroid lipofucinosi

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Purpose To describe the electroretinographic and ultra-structural alteration in a very rare family case of Neuronal Ceroid Lipofucinosi (NCL).

Methods A 7-year-old boy and his brother a 5-year-old boy with NCL of juvenile type, underwent complete ophthalmological exams, including retinography, electroretinography and ultra structural study of the conjunctiva.

Results Both children presented optic nerve pallor and arteriolar narrowing. The funduscopy of the oldest boy showed bone-spicule changes. The ERG scotopic (rod and maximal) and photopic (cone) responses were absent. The flicker responses were absent in the oldest brother and severely reduced in the youngest brother. It could be a typical case of retinitis pigmentosa. The electron microscopy study of the conjunctiva, detected the curvilinear bodies typical from Neuronal Ceroid Lipofucinosi (NCL).

Conclusion The retinal and electroretinographic changes were typical from retinitis pigmentosa. The electron microscopy study is an important tool in LCN diagnosis.

■ 3324

Accommodation and pupillary response in the reading of texts with varying cognitive demand

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Purpose The relationship of the pupil size changes and lag of accommodation during reading of texts with varying cognitive demand was examined to determine whether the content of reading has significant influence on the accommodative and pupillary response. The results are discussed in regard to possible impact on myopia growth.

Methods The study included 112 high school students (84 female, 28 male) aged 17 to 19. At the distance of 40 cm (≈ 2.5 D) five short texts with different cognitive demands were presented on a laptop screen. From 1.0 m distance, the measurements of pupil size and accommodation were taken using the Plusoptix Power Refractor.

Results Accommodative lag was found to be very stable during all the tests, with the mean value of 0.275 D. On the other hand, the pupil size varied significantly, depending on the mental effort and type of cognitive involvement. Smallest pupils were associated with the reading of advanced philosophically blended texts (4.79 mm), whereas texts with simple calculations were associated with greatest pupil size (5.08 mm). The difference between these two was statistically significant with $p = 0.0024$.

Conclusion Young subjects reading contents with higher cognitive demand, particularly those that require instant reasoning, have larger pupils than subjects reading philosophical novel-like texts. Larger pupils are associated with more exaggerated aberrations and smaller depth of focus. With consistent lag of accommodation and considering accommodative microfluctuations, retinal image in this case must be blurrier or at least closer to the blur point. It can be concluded that the content of reading itself is a factor in nearpoint stress and could hypothetically contribute to myopia growth.

■ 3325

Prevalence of myopia in preschool Singapore Chinese children

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Purpose To determine the prevalence of refractive error types in Singaporean Chinese children aged 6 to 72 months.

Methods The Strabismus, Amblyopia and Refractive error in Singaporean Children (STARS) is a population-based study conducted in southwest Singapore. Door-to-door recruited participants were selected using a disproportionate random sampling in 6 month increments. Eye examinations included LogMAR visual acuity, cycloplegic autorefractometry, and IOL Master biometry. Overall and age-specific prevalences of myopia (spherical equivalence [SE] < -0.50 diopters [D]), high myopia (SE < -6.00 D), hyperopia (SE > +3.00 D), astigmatism (cylinder > +1.50 D), and anisometropia (SE difference between each eye > 2.00D) were calculated.

Results 3009 children were examined (participation rate 72.3%). Right eye (OD) cycloplegia data were available for 1375 boys and 1264 girls. Mean OD SE was +0.69D (SD 1.15D). Overall myopia prevalence was 11.0% with no gender variance ($p = 0.91$). High myopia prevalence was 0.2%. Prevalences of hyperopia, astigmatism and anisometropia were 1.4%, 8.6%, and 0.6%, respectively. Myopia was present in 15.8%, 14.9%, 20.2%, 8.6%, 7.6%, and 6.4% of children aged 6-11, 12-23, 24-35, 36-47, 48-59, and 60-72 months, respectively. Astigmatism prevalence increased with age ($p < 0.001$), but hyperopia and anisometropia did not, $p = 0.55$ and $p = 0.37$, respectively.

Conclusion Prevalences of myopia and astigmatism in Singaporean Chinese preschoolers are high, and low for hyperopia. Age effects were observed for each refractive error category.

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Comparison of refractive cylinder and corneal cylinder in aphakic eyes

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Purpose This paper compares the refractive cylinder in aphakic eyes measured with an intraoperative wavefront aberrometer to preoperative corneal cylinder values which have been corrected for an assumed magnitude of induced cylinder on the axis of the phaco incision. Such a difference could play a significant role in planning corrective cylinder procedures such as LRIs or Toric lens positioning during cataract surgery.

Methods Preoperative corneal cylinder was measured for 30 eyes undergoing standard cataract surgery. Using established cross cylinder calculation methods and assuming an induced cylinder of 0.5 D at the phaco incision axis, a modified corneal cylinder was calculated. These modified values were compared to aphakic refractive cylinder values measured with the ORange intraoperative wavefront aberrometer. These aphakic measurements were obtained with the globe re-inflated with viscoelastic to an IOP of 20 - 25 mm Hg. Three aphakic measurements were obtained for each eye and averaged.

Results The modified corneal cylinder should be nearly equal to the aphakic refractive cylinder if the anterior corneal surface represents the total corneal contribution to astigmatism as any affect of the natural crystalline lens on the refractive cylinder has been removed. Statistical differences were found between the modified corneal cylinder (from preoperative keratometry and induced cylinder) in ~15% of the 30 eyes. Differences occurred in both the magnitude and axis of the astigmatism.

Conclusion The use of an intraoperative wavefront aberrometer can significantly improve the outcomes of cylinder reducing procedures such as LRIs and Toric IOLs by correctly identifying the magnitude and axis of the patient's refractive corneal astigmatism during surgery.

Commercial interest

■ 3331

Central corneal thickness (CCT) and intraocular pressure (IOP) in myopic adult Chinese

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Purpose To investigate CCT and IOP in myopic adult Chinese and to estimate its relationship with age, refraction, sex before excimer surgery in Eye Department of Daqing People's Hospital (the 5-th Affiliated Hospital of Harbin Medical University).

Methods We studied a series of 402 healthy eyes of 201 patients (76 male /37.8%, 125 female /62.2%) who underwent excimer surgery for the correction of myopia with the sphere up to -13.50 D and the cylinder to -4.50 D. All ophthalmic investigations were made including pneumotometry ("Canon" TX-10 and T-F) and ultrasound (US-) corneal pachymetry with CCT-measurements ("Nidek" UP-1000). We performed accurate statistical analysis by using "Statistica" program.

Results The age of our patients was 26.5+/-6.3 years. UCVA and BCVA of all patients were 0.13+/-0.09 and 0.97+/-0.09 consequently. The results showed that mean US-CCT of our male patients was 548.03+/-29.12 microns. But mean CCT of our female patients was 537.77+/-32.09 microns, 10.26 microns less than that of male patients ($p < 0.01$). The mean IOP of male participants was 16.23+/-2.94 mm Hg, and mean IOP of female participants was 14.82+/-2.74 mm Hg ($p < 0.01$).

Conclusion Our results confirmed some data (P. Li et al., 2006) that mean US-CCT of male patients was statistically significant more than such measurements in female ones in myopic adult Chinese. We revealed that pneumotometric IOP was positively related to US-CCT ($r = 0.85$). None of the authors has no financial interest in this work.

■ 3333

Intraocular pressure measurements values in patients who have had uneventful deep anterior lamellar keratoplasty

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Purpose To effect of central corneal thickness (CCT) and graft site thickness (GS) in patients who have had uneventful deep anterior lamellar keratoplasty (DALK) using "big bubble" technique, on intraocular pressure (IOP) measurements, investigated using two different techniques. Retrospective cross sectional study from May 2008 to April 2009 on Ophthalmology Department of District Railway Hospital in Katowice, Poland.

Methods Patients 40 after DALK, at least 12 months prior to testing. Control group: 30 patients. IOP was determined by Goldman applanation tonometer (GAT) and iCARE (IC) rebound tonometer. CCT and GS were assessed by Anterior Segment OCT (Visante OCT). Mean GAT and IC IOP were calculated for: DALK and control. Agreement between results from IC and GAT were assessed using the Bland-Altman method. The deviations of IC readings from GAT, corrected for CCT were calculated using a linear regression model.

Results In DALK mean CCT was 470.6 +/- 39 μ m and in control 550.9 +/- 29 μ m. Mean IOP +/- SD in DALK 16.5 +/- 11.1 mmHg in control 16.7 +/- 4.2 mmHg for IC. Mean IOP using GAT for studied group was 15.7 +/- 2.4 mmHg and for control 13.2 +/- 2.6 mmHg. IC IOP was significant higher than GAT ($P < .005$). Difference between IOP and CCT and GS were not statistically significant ($P = .53$). Correlations between IOP and CCT were not significant in these cohorts ($P > .09$). Agreement between IC and GAT was assessed.

Conclusion IOP measured by IC was consistently higher when compared with GAT, this difference was greatest with thicker CCT but not with thicker GS. Falsely underrated IOP in DALK was not demonstrated.

■ 3332

Validation and use of simulated corneal endothelium specular microscopy images

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Purpose The aim of the current study was to develop simulation software capable of producing life like images of the corneal endothelium (CE) captured by clinical specular microscopy (CSM). Exact morphological data would come with each image. Once evaluated for authenticity, simulated images would be used for evaluation of the precision of methods of morphometry of corneal endothelium density (CED).

Methods Software for simulation of images of the CE captured by CSM was developed. Several unique images of the CE were created, spanning a range of CED. 12 images, evenly spread over a CED range of 1959-3838 cells/mm² were selected. The images were uploaded to a PC with border tracing software for CE morphometry. The semi-automated morphometry software was used to analyze the CED of the 12 images. The CED results given by the analysis and the CED given from the creation of the images were analyzed by linear regression.

Results An expected correlation between CED given from the creation of the simulated images and the CED given from the semi-automated analysis was found. Assuming CED given by the model were exact, the standard deviation of the residual variance of the analyzed CED was estimated as a 95% confidence interval to 15.8-91.0 cells/mm², corresponding to a variation coefficient of 0.5-3.1%. Average CED of the 12 samples was 2899 cells/mm².

Conclusion A simulator capable of realistic CE structures is a useful tool. Not only can it be used to estimate the accuracy of current methods of CE morphometry – it can also be used for development of new methods of morphometry, due to the virtually unlimited access to unique realistic images that come with exact and pre-defined morphological data.

■ 3334

Technical standardisation to measure the ocular surface lipid layer with Meibometer 550

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Purpose Evaluation of measuring errors in usage of the meibometer 550.

Methods Measurements of lipid layer of the ocular surface tear film were done by Meibometer 550. For exact measurement we observed possible disruptions, the calibration, hygienically standards, different sampling, alteration after topical treatment. Before each measurement there must be a calibration by clean lipid -free stripes. We observed the adjustment with two clean stripes by double measurement of each other. Both results must be 0. Hygienically influence is presumed by incorrect handling if objects are touched. Stripes were pressed on objects and measured. Those objects were the table surface, the hand of the observer, the gloves and the disinfectant used. We observed different pressure sampling as pressure may extract more lipids from the lid with touch of the lid and no touch, with pressure and without. For the influence of topical treatments we dropped several artificial tears directly on the stripes, watery artificial tears, gels, ointments and drops with lipid portions.

Results After double calibration new stripes showed values of nearly 0, elder stripes 10-18 u. Hygienically: in clean tables we measured <28u, even clean hand <60 u, gloves <5u. Disinfectant fluids decreased measured lipids from 500 to 100 u. There was a difference in sampling with pressure to samplings without pressure between 50 and 80%. Topical treatments changed different after use of watery drops: 0-15u, lipid spray: change 370 u average, fatty ointments: change of 557u to 751 u.

Conclusion It is important to notice the careful handling during measuring the lipid layer with MB 550 to obtain valid measuring data.

Commercial interest

■ 3335 / 306

A pocket slitlamp

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Purpose I here describe a prototype pocket slitlamp for students and non-specialists. It might help bridge the growing gap between ophthalmology (and optometry) and other specialties.

Methods MATERIAL Pocket microscopes, condensing lenses, modified pentorch and a platform. PILOT TESTS Evaluation and photographs of optical sections of 1) Anterior segment and lens of the eyes of the author and four volunteers. 2) Wrist watch crystals. Is a wristwatch a suitable model eye?

Results The prototype weighs 100g, costs EUR35 (£30) and can be carried in a pocket. The magnification was 0-30X, and the slits of light were 0.5-2mm wide. The digital camera cost EUR85 (£79.99). PILOT TEST RESULTS 1) Clinically adequate optical sections of the cornea, anterior chamber and lens were seen and photographed. 2) The outer and inner surfaces of the wristwatch crystal, watch face and the space in between were clearly seen. They simulated the corneal epithelium and endothelium, the iris and the anterior chamber. LIMITATIONS The view is monocular, there is no zoom and the light is less intense than the maximum of a conventional slitlamp.

Conclusion When developed this miniature slitlamp could help both in the instruction of students and in the clinical practice of non-specialists. Students could practice on each other and on wristwatches, which make good model eyes.

Commercial interest

■ 3337 / 308

Simultaneous topo-guided photorefractive keratectomy (PRK) followed by collagen cross-linking (CXL) for the treatment of keratoconus

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Purpose To present the results after simultaneous topoguided Photorefractive Keratectomy (PRK) followed by corneal collagen cross linking with Riboflavin/ Ultraviolet – A irradiation (CXL) for the treatment of keratoconus.

Methods In this prospective case series, 22 patients (27 eyes) with progressive keratoconus participated. All patients underwent customized topography guided photorefractive keratectomy (PRK) immediately followed by corneal collagen cross linking with the use of riboflavin and ultraviolet – A irradiation.

Results Mean follow up was 10.72±5.98 months (range from 3 to 19 months). Mean preoperative spherical equivalent (SE) (diopters, D) and defocus were -3.03 ± 3.23 D and 4.67 ± 3.29 D while at the last follow up examination were significantly reduced to -1.29 ± 2.05 D and 3.04 ± 2.53 D, respectively. Preoperative mean (LogMAR) Uncorrected Visual Acuity (UCVA) and Best Spectacle Corrected Visual Acuity (BSCVA) were 0.99 ± 0.81 and 0.21 ± 0.19 while at the last follow up examination were improved to 0.16 ± 0.15 and 0.11 ± 0.15 respectively. Mean preoperative steepest meridian keratometry from 48.2 ± 3.4 D was reduced to 45.13 ± 1.8 D at the last follow up examination.

Conclusion Simultaneous PRK followed by CXL seems to be a promising treatment capable of offering patients a functional vision and halting progression of the ectatic disorder. Longer follow up and larger case series are necessary in order to fully evaluate this new innovative combined procedure.

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Dextran 70 solutions for the intra-operative control of corneal hydration in rabbit corneas

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Purpose In terms of excimer laser ablation, the cornea is water bound on an organic matrix. Corneal hydration might affect the excimer laser ablation rate, which could affect the accuracy of correction. It was the purpose of the study to investigate the use of Dextran solutions of varying concentrations to control corneal hydration in rabbit corneas.

Methods The corneal epithelium was removed by means of a rotating brush from both eyes of 10 anesthetized pigmented rabbits. The Dextran 70 solutions, with concentrations ranging from 1 to 7%, was topically applied to the eyes for two minutes. The thickness of the cornea in each eye was determined by ultrasound pachymetry after epithelial removal and immediately after rinsing. The Dextran concentration corresponding to zero change in pachymetry was determined by linear regression. In a different series of experiments, rabbit corneas were either overhydrated by means of rinsing with saline or left exposed to dehydrate. Following this artificial change in hydration the corneas were rinsed for 2 minutes with the previously determined iso-osmotic concentration.

Results A strong negative correlation between Dextran concentration and change in pachymetry was observed. Rinsing with the isotonic concentration (2.6%) regulated pachymetry within 2% of the original value.

Conclusion Corneal hydration can be regulated intraoperatively by means of appropriate Dextran solutions. This may prove useful in cases that hydration is potentially different from normal such as in the case of extensive surgical manipulations prior to laser ablation.

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Comparison of morphometric and morphological OCT Visante and UBM potential in anterior segment imaging

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Purpose To compare anterior segment modalities such as Visante OCT and UBM as an important tool for assessing anterior segment examination.

Methods 166 patients (96 women and 70 men, mean age 54.32 ± 9.92 years) various pathologies in anterior segment area and after refractive and glaucoma surgery were examined with both OCT Visante (Zeiss) and UBM (QuantelMedical). In the above examination, we have compared sectorial b-scan image, made by 50Hz UBM head (patient in horizontal position) to scan image displaying anterior chamber (ac-scan), made by OCT Visante device (patient sitting).

Results Both devices promise quantitative information and imaging of corneal pathologies, keratoplasty results and follow up also anterior chamber imaging, direct angle visualization, lens pathologies, diagnostic and postoperative results of cataract and glaucoma. Bland&Altman analysis has shown, that results obtained by UBM in comparison to Visante OCT in anterior chamber depth measurement (AC) were averagely higher by 0.21mm (± 1.96 SD= 0.30) in case of PKP and 0.2mm (± 1.96 SD= 0.23) in case of lamellar keratoplasty. The results for filtration angle measurement, were averagely higher by 3.3 degrees (± 1.96 SD= 4.30) in case of PKP, and 3.4 degrees (± 1.96 SD= 3.9) in case of lamellar keratoplasty. Calculations obtained by ultrasound biomicroscope were slightly higher, however there was no significant statistic difference.

Conclusion Both used devices are helpful in clinical practice, however OCT Visante, with high resolution scans and as a non contact device with shorter examination time is more usefull in every day practice. Moreover UBM is an indispensable tool for anterior segment tumors involving ciliary body.

■ 3341

Antioxidants reduce diabetic damage in bovine lenses in culture

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Purpose Background: There are several theories regarding possible mechanisms leading to diabetic cataract. Few of them include oxidation stress. Aims: Investigation of the mechanisms of cataract formation under diabetic conditions, and examination of the effects of N-acetyl-L-cysteine (NAC), (which is a precursor of glutathione and an anti-inflammatory agent) and derivatives of Desferrioxamine (DFO) (which is an iron chelator and reduces oxidative stress) on diabetic cataract.

Methods The experiments included 78 bovine lenses. The lenses were divided into eight different treatments including controls and lenses incubated with high glucose levels (450 mg %) with or without each one of the antioxidants. The intact lenses were incubated for a period of two weeks in our special organ culture conditions. Lens optical quality was analyzed every 24 hours. At the end of the culture period, oxidation was followed in the lens epithelial cells with dichlorofluorescein assay and lens proteins were analyzed by SDS and 2D gel electrophoresis.

Results High levels of glucose in the culture medium caused optical damage to bovine lenses, increased lens volume due to swelling, increased oxidation of lens epithelial cells, and caused changes in lens beta crystallin. The anti-oxidants reduced this damage. NAC and Zn-DFO protected the lenses better than DFO.

Conclusion Antioxidants can protect the lens from high glucose damage. This study was supported in part by a grant from the Esther and Chaim Coppel Trust and by the Guzik Ophthalmology Research Fund

■ 3343

Nanostructural properties of intraocular lenses (IOLs) – atomic force microscopy (AFM) and Fourier transform infrared spectroscopy (FTIR) investigations

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Purpose To investigate and analyze surface of explanted intraocular lenses (IOL-s), by means of the Atomic Force Microscope (AFM) and Fourier transform infrared spectroscopy (FTIR).

Methods Eight dry IOL-s (acrylic: SN60AT – 3 pieces, SA60AT, CZ70BD; silicone: CLRFLXC; "hard": AJPR,CP65T), and four hydrophilic acrylic IOL-s (AC-IOL and PC-IOL, both: naïve and removed during keratoplasty) were imaged (topography and phase) with AFM. AFM is used to investigate, at nanoscale, the surfaces' topography and some nanomechanical properties (eg. elasticity, hardness) of materials in medicine. The samples can be investigated in quasi-physiological conditions, usually no damaging preparation is required. For the acrylic and "hard" lenses the granular nanostructure is observed. The roughness of the lenses' surfaces can be then assessed. The silicone lens is soft: the forces of the nanonewtons applied during imaging cause strong deformations of the material.

Results Nanomechanical properties of PC-IOL lens are not uniform. This may be advantageous for its calcification: for the lens of the same type removed during keratoplasty strong calcification is observed. For SN60AT lens the influence of the application process (folding) on its nanostructure is tested. The observed structural nano-defects are permanent, they can occur during folding or can be caused by the used equipment. The similar nano-deformations are observed for the removed AC-IOL lens.

Conclusion AFM and FTIR showed to be a high-resolution imaging tool for the scanning of surface IOL.

■ 3342

Cell death in lens epithelial cells after stimulation of the sigma-2 receptor

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Purpose The aim was to investigate the mechanisms of cell death in lens epithelial cells after administration of siramesine, a sigma-2 receptor agonist.

Methods Human lens epithelial cells in culture were exposed to siramesine and examined for morphological changes using DIC or calcein as a cytoplasmic marker. Lysosomes were studied using acridine orange and MagicRed. Proteolytic activity of the proteasome, calpain, caspases and cathepsins in living cells or cell extracts were studied using different fluorogenic substrates.

Results Siramesine at low concentrations increased the cytoplasmic proteolytic activity of the proteasome and the calpain system. Early effects was also observed with respect to lysosomal morphology, acidity and function. Activation of caspase-3 and the appearance of nuclei with an apoptotic morphology were also found.

Conclusion Siramesine at very low concentrations affects lens epithelial cells with perturbation of the major proteolytic systems, lysosomal morphology and results in caspase activation and cell death. Siramesine may be a promising substance for clinical studies concerning the treatment of PCO.

■ 3344

Predictive factors of visual outcome in acute post-cataract endophthalmitis

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Purpose To study potential clinical and microbiological predictive factors of visual outcome in patients with acute endophthalmitis following cataract surgery.

Methods A prospective study included 100 patients in 4 University hospital. Factors related to the cataract surgery, the initial clinical presentation and the microbiological identification were analyzed according to the final visual outcome using univariate and multivariate (logistic regression) analysis.

Results 46% out of the patients had a final visual acuity less than or equal to 0.3 logMar (good visual outcome) at 6 months while 10% had only light perceptions. Patients with good visual outcome differed for the duration of cataract surgery, initial visual acuity, the visibility of fundus and the identification of a coagulase negative staphylococcus. In contrast, patients with a poor visual outcome were older, had more cornea oedema and a more important hypopyon at the admission, more complications at the time of cataract surgery. Furthermore a bacterium was more frequently identified in this latter group. Multivariate analysis showed that age, complications at the time of cataract surgery, microbiological identification, pars plana vitrectomy were independent predictive factors.

Conclusion Factors of visual outcome in acute postcataract endophthalmitis identified in this prospective study were similar to that reported during the Endophthalmitis Vitrectomy Study 10 years ago. As part of the treatment, pars plana vitrectomy is associated with predictive factors of poor visual outcome. Identification of these predictive factors at presentation should allow a better management of patients needed an aggressive treatment.

■ 3345

The crystalline lens higher fatty acids influence on the lens physical characteristics

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Purpose To study influence lipids of a crystalline lens on its physical characteristics.

Methods 114 patients at the age from 55 till 78 years, with a cataract of various degree of a maturity are selected. Researches of a lens nucleus in vitro included: definition of mechanical hardness on the original device for research of mechanical hardness of a crystalline lens, research of composition of the higher fatty acids on the gas-liquid chromatograph.

Results Influence of the higher fatty acids composition on mechanical hardness of a lens nucleus is revealed. Strong direct dependence between polyunsaturated fatty acids contents and hardness of a crystalline lens is found out. The correlation factor is equal 0.7 ($p < 0.05$). Pentadecanic and stearic acids - the saturated fat acids have average correlation with hardness of lens - the correlation factor is accordingly equal -0.6 ($p < 0.05$) and -0.6 ($p < 0.05$), in spite of low contents in the lens. The palmitic acid contains in the cataract lens in concentration comparable to level polyunsaturated fatty acids, however, essential correlation with hardness has no - the correlation factor is equal 0.21 ($p > 0.05$). The oleic, linolic and linolenic acids are contained in the cataract lens in low concentration and have average (v and linolic acids) - the correlation factor is accordingly equal -0.5 ($p < 0.05$) and -0.57 ($p < 0.05$) and weak correlation (linolenic acid) with hardness of a crystalline lens - the correlation factor is equal -0.22 ($p > 0.05$).

Conclusion The polyunsaturated higher fatty acids which are a basis of membranes of a crystalline lens have the maximal influence on mechanical characteristics of the lens.

■ 3346

Hardness of a crystalline lens and accommodation

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Purpose to estimate influence of mechanical characteristics of a crystalline lens on accommodation possibility

Methods At 56 patients with a various condition of a crystalline lens we defined amplitude of accommodation by a method of finding of the nearest and far points. Mechanical properties of a crystalline lens defined by the original ultrasonic method. At the heart of a method, use of the information which formed at distribution of a mechanical wave in non-uniform environments.

Results We revealed the strong inverse negative relationship between volume of accommodation and hardness of a crystalline lens nucleus - the factor of correlation was equal -0.63 ($p < 0.01$).

Conclusion One of the reasons of the accommodation worsening is the increase of a crystalline lens mechanical hardness.

■ **3351**
Evaluation of quality of life, and priorities in people with glaucoma

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Purpose Quality of life appears to be of increasing importance as a criterion for clinical intervention. However its meaning can be complex and its assessment varied. In social science the term has broad definitions which include terms such as autonomy, wellbeing; self esteem; sense of control etc. On the other hand within ophthalmology a narrower operational definition is mainly used which is the degree to which someone's vision impacts on a range of necessary and desirable daily tasks a person wishes to carry out. The purpose of the presentation is to compare alternative methods of quality of life assessment.

Methods The assessment approaches taken in the study range from conventional questionnaire rating scales, (something NICE has questioned) and time trade off comparisons, to more recent methods of scaling generated by for example Rasch or Hierarchical Bayesian analysis.

Results Data will be presented from two studies (one in Edinburgh and one in Aberdeen) on quality of life in people with glaucoma. One of the new recommended discrete choice methods (Choice based conjoint analysis with Hierarchical Bayesian estimates) will be used. The results will include quality of life outcomes and their stability; related visual factors; comparisons across methods and more general implications for quality of life assessment.

Conclusion Different methods for the assessment of quality of life produce different results with relatively low correlations between them although conjoint analysis has revealed stable priorities across two independent studies. These discrepancies in quality of life assessment require further study and evaluation.

■ **3353**
What do patients with glaucoma do when they search and look at everyday scenes?

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Purpose To test the hypothesis that patients with bilateral glaucoma exhibit different eye movements compared to normally-sighted subjects when viewing computer displayed photographs, or searching for features or items within them.

Methods Thirteen glaucoma patients and 17 age-matched subjects with normal vision viewed 28 randomised digital photographs of various everyday scenes displayed on a computer screen for 3 seconds each. Subjects were instructed to view the images as they would when looking at a slideshow. The subjects then viewed another set of images, but were timed to find a feature or item in the scene. Eye movements were simultaneously recorded using an Eyetracking system.

Results In the passive viewing experiment, there was a significant reduction in the average number of saccades for glaucoma patients compared to controls ($p < 0.0001$). In addition, average fixation duration was longer and the average area scanned was more restricted in patients compared to controls. In the search task glaucoma patients took, on average, longer to find the objects ($p < 0.0001$) compared to controls. For this task, saccades were still reduced in number. In some cases, individual scanning patterns appeared related to the type and nature of the binocular visual field defect.

Conclusion Eye movement behaviour in patients with glaucomatous defects in both eyes differ from normal-sighted subjects when viewing images and photographs. These patients with glaucoma find it more difficult to locate items within scenes compared to normally sighted subjects. Acknowledgements: This work is generously supported by an unrestricted grant from the Special Trustees of Moorfields Eye Hospital.

■ **3352**
Measuring visual diasability in glaucoma

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Quantification of the visual disability experienced by the glaucoma patient is currently limited to the use of clinician-based measures of disease status; that is, measurement of retinal nerve fibre layer thickness, visual field sensitivity and intraocular pressure. However, whilst these tests provide information to the clinician regarding patient management requirements, they provide very little information about the patient's ability to function on a day-to-day basis. There have been attempts to measure the glaucoma patient's experience using vision-specific 'quality of life' questionnaires. However, it is apparent that no two patients with the same disease status will report the same quality of life experience. The purpose of this talk is to present new work examining how glaucoma patients perform specific, day-to-day tasks in the presence of the disease, with a view to understanding what specific visual factors might explain the difficulties they encounter at different stages of the disease.

■ **3354**
Fitness to drive in glaucoma patients- Preliminary study results

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Purpose To develop a useful binocular 30° visual field criterion to predict safe driving behaviour in glaucoma patients by comparing perimetric data with an actual driving test on the road.

Methods The sample will consist of 200 driving glaucoma patients, recruited in 2 university based glaucoma clinics (Ghent and Leuven, Belgium). Inclusion criteria are glaucomatous optic disc damage and/or glaucomatous field defects. Exclusion criteria are concomitant ocular disease, cataract > LOCS 2, systemic disease or medication affecting the visual field. Data collection will include demographic and medical data, driving habits, and Mini Mental Status. A complete ophthalmic examination will be done including Goldmann, SAP and Esterman visual field testing. In addition, UFOV test, stereopsis and contrast sensitivity testing will be performed. All subjects will perform an on the road driving test with a driving expert of the Belgian Institute for Traffic Safety. Subjects can pass, fail, or pass the test with limitations. An attempt will be made to develop an algorithm of visual field abnormalities that predict as accurately as possible the outcome of the practical driving test.

Results Preliminary results of the first 50 included patients will be presented.

■ 3361

IC3D classification of corneal dystrophies

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Conclusion The IC3D group, an international group of corneal specialists and ophthalmic pathologists, formed a few years ago to review known corneal dystrophies. Based on personal experience and literature studies the new IC3D classification of corneal dystrophies was published in December 2008 as a freely available supplement to the journal *Cornea*. The classification suggests new names for some dystrophies, suggests definitions when an entity could be called a dystrophy, and gives examples of typical slit lamp images. It is built in a way to accommodate new and variant dystrophies found in the future. The purpose of this symposium is to draw attention to this new classification with special emphasis on recent variant dystrophies associated with the TGFBI gene. The IC3D group urges authors and reviewers alike to require stringent criteria to be met before publication of a dystrophy as a new entity.

■ 3363

Overview of the TGFBI corneal dystrophies

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Several phenotypically distinct clinicopathologic entities involving the cornea are caused by mutations in the transforming growth factor beta induced (TGFBI) gene. These disorders include different types of granular corneal dystrophy (GCD): GCD type 1, GCD type 2 (Avellino corneal dystrophy), GCD type 3 (Reis-Bücklers corneal dystrophy) as well variants of lattice corneal dystrophy type 1 and Thiel-Behnke corneal dystrophy. Investigations of these inherited corneal diseases throughout the world strongly suggest that specific mutations in the TGFBI gene account for the specific phenotypes and that the corneal opacities that account for the clinical features of the different phenotypes result from the deposition of all or part of the mutated encoded protein. To date the mutated protein is only known to accumulate in the cornea even though the TGFBI is widely expressed throughout the body in experimental animals. This presentation will provide an overview of the TGFBI corneal dystrophies and offer a hypothesis to explain the different phenotypes caused by different mutations in TGFBI.

■ 3362

How to tell dystrophies of Bowman's layer (Reis-Bücklers and Thiel-Behnke) from one another

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Purpose To discuss difficulties encountered in differentiating two classic dystrophies of the Bowman layer caused by mutations of the TGFBI gene: Thiel-Behnke corneal dystrophy (TBCD) and Reis-Bücklers corneal dystrophy (RBCD).

Methods Critical review of published data by the IC3D group and personal experience of the author as an ophthalmic pathologist and clinician.

Results It has traditionally been difficult to recognise and distinguish TBCD and RBCD, so much so that some early papers claimed to describe the ultrastructure of RBCD actually analysed tissue from patients with TBCD; the known entity of RBCD was described as an unusual variant of granular corneal dystrophy; and Waardenburg-Jonkers dystrophy appeared for years in textbooks although their patients had TBCD. The main problem is that both dystrophies are fairly rare and clinicians seldom have personal prior experience or adequate literature knowledge of both when they encounter a patient; and the literature with its frequent errors in diagnoses has not been that helpful.

Conclusion TBCD and RBCD may be differentiated by careful clinical and ophthalmic pathologic examination if the clinician and pathologist are experienced; genetic or electron microscopic examination is needed for unequivocal confirmation.

■ 3364

A variant TGFBI corneal dystrophy from G623D mutation with an unusual amyloidogenic phenotype

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Purpose To present a unique corneal dystrophy never before described in a German family carrying the Gly623Asp mutation of the TGFBI gene with late clinical onset.

Methods Clinical documentation and isolation of genomic DNA from peripheral blood leucocytes were obtained from each family member examined. Exons 3, 4, 5, and 11 to 14 of the TGFBI gene were sequenced. 5 corneal buttons of 3 affected siblings were excised at the time of penetrating keratoplasty. Light and electron microscopic examination were performed including immunohistochemistry with antibodies against Keratoepithelin (KE) 2 and 15.

Results Specimens showed changes in Bowman's layer and the adjacent stroma. Congo red-positive amyloid deposits were found within the epithelium in one cornea, in Bowman's layer and in the anterior stroma of all specimens, also showing KE2- but not KE15-immunostaining. EM revealed deposits located in the anterior stroma and Bowman's layer and the basal area of some epithelial cells. These areas were strongly Alcian blue-positive but negative in the Masson-Trichrom-stain. Only affected patients had a heterozygous missense mutation in exon 14 of the TGFBI gene (G->A transition at nucleotide 1915) with the change Gly623Asp in the keratoepithelin protein.

Conclusion In contrast to the patient carrying the Gly623Asp mutation of the TGFBI gene described by Afshari et al., our cases presented with Salzmann nodular degeneration like clinical features with KE2 positive amyloid. The reason for this now "meeting the expectation histological phenotype" is unclear. The histological findings emphasize that this is a unique corneal dystrophy which shares no clinical characteristics with Reis-Bücklers dystrophy and should be treated as a distinct entity.

■ 3365

TGFBI gene mutations in Hungary – polymorphic corneal amyloidosis caused by the novel F547S mutation

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Purpose To identify mutations in the Transforming Growth Factor Beta Induced (TGFBI) gene in Hungarian patients with corneal dystrophy and to characterize their histological features.

Methods Exons of TGFBI gene were sequenced in 38 members of 15 unrelated families with corneal dystrophy. Exon 12 was sequenced in 100 healthy controls. Immunohistological analysis of corneal buttons excised during penetrating keratoplasty was performed.

Results Molecular genetic analysis revealed a heterozygous R124C mutation in 18 patients with lattice type 1 dystrophy. A R555W heterozygous mutation was detected in five patients with granular Groenouw type I corneal dystrophy and the R555Q heterozygous mutation was found in four patients clinically diagnosed with Reis-Bücklers (one patient) and Thiel-Behnke (three patients) dystrophy. Three patients with "atypical granular" dystrophy later diagnosed as Avellino dystrophy were heterozygous for the R124H mutation. No other than the novel heterozygous T1640C mutation causing the F547S amino acid exchange was detected in a patient with polymorphic corneal amyloidosis. The mutation could not be found in healthy controls. Immunohistochemistry showed the presence of BIGH3 protein deposits in all examined buttons. Electron microscopy confirmed the presence of amyloid fibrils in the case of the novel mutation.

Conclusion Our results indicate that molecular genetic analysis is required to confirm the diagnosis of corneal dystrophies. We report the first cases of Avellino dystrophy from Central-Eastern Europe. The novel F547S mutation causes polymorphic corneal amyloidosis.

■ 3411

New perspectives of optical coherence tomography in diagnosis and follow-up of macular holes

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Purpose To compare Time Domain (TD) with Spectral Domain (SD) OCT for imaging macular holes, identify retinal pathology and correlate anatomical morphology after surgical intervention for hole closure with visual outcome.

Methods 34 eyes of 34 patients with idiopathic macular holes stage II-IV were included in this study. Comparative studies were performed with both SD OCT (Heidelberg, Germany) and TD OCT (Stratus) using standard scanning protocols of 6 radial 6-mm scans through the fovea. All patients underwent a standard three-port pars plana vitrectomy. Postoperatively, all patients were evaluated using both OCTs. ETDRS visual acuities were recorded pre- and post-operatively.

Results In general TD and SD OCTs showed comparable images of macular holes. However, the boundary line between the inner and outer segments of the photoreceptors was better imaged with the SD OCT preoperatively and postoperatively. Poor visual acuity postoperatively was measured mainly in cases with morphological disruption in this boundary line despite hole closure.

Conclusion SD OCT imaging enhances the visualization of retinal anatomy in macular holes relative to TD OCT.

■ 3413

Internal limiting membrane staining

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Purpose To describe the different modalities of internal limiting membrane (ILM) staining for the treatment of idiopathic macular hole (IMH).

Methods Search of the MEDLINE database by using Medical Subject Heading search terms and key words related to ILM staining, macular hole surgery.

Results ILM removal has been closely related with increased closure rates. However, because of poor ILM visibility the surgical removal of ILM is very difficult and poses serious complications. To obtain better visibility of ILM some special techniques have been developed using specific dyes such as, trypan blue (TB), indocyanine green (ICG), infracyanine green or triamsinolone acetate (TA). Anatomic success rates with one surgery have increased (>90%) however, concern for toxicity has emerged. Patients with ICG-assisted ILM peeling appear to have a depressed recovery of visual acuity compared to those not using ICG. Concentrations of ICG that are <0.5 mg/mL have been shown to be non-toxic in cultures of RPE cells. Infracyanine green is a similar molecule that does not contain iodine and is less likely to induce osmolarity related toxic effects on the PRE cells when compared to ICG. It has been used also for ILM staining in combination with trypan (TB). TB is a second generation vital dye that stains epiretinal membranes (ERMs) directly and ILM to a lesser extent. Thus TB is useful for both macular hole and macular pucker surgery. TA may be also used to help highlight the ILM, although it is not a dye. It does adhere to the posterior hyaloid, making the detection of ILM easier.

Conclusion This review largely reflects the great advent of different techniques for ILM staining. The use of ICG is more toxic when compared with TB. For TA long-term effects have not been well studied.

■ 3412

Macular hole surgery with and without internal limiting membrane peeling

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Purpose To compare the results of surgery for idiopathic macular hole with or without the surgical removal of the internal limiting membrane (ILM) and the effect on anatomical and functional success rates.

Methods 41 consecutive patients with idiopathic macular hole stage II (n=11), III (n=22) and IV (n=8) underwent pars plana vitrectomy and intraocular gas tamponade in this study. The surgery was performed either with ILM peeling, Group A (n=28), or without, Group B (n=13). Ocular coherence tomography and ETDRS visual acuity were measured pre- and postoperatively to assess macular hole size and anatomical hole closure as well as visual function.

Results The postoperative macular hole closure rate was 100% (28/28) in Group A and 84.6% (11/13) in Group B. The 2 cases that failed to close with primary surgery had a hole size greater than 400µm. There was no significant difference between the two groups in the postoperative visual outcome once anatomical success was achieved.

Conclusion The study suggests that more evidence based trials are necessary to investigate the benefit of ILM peeling especially in the treatment of larger macular holes.

■ 3414

23G versus 20G for macular hole surgery. Efficacy, safety and patient satisfaction

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Purpose To compare the 20gauge and 23gauge vitreoretinal surgical system and equipment in macular hole surgery.

Methods We randomly assigned 38 patients in two different groups. Group 1. Used 20g surgical equipment and instrumentation. Group 2. Used 23g surgical equipment and instrumentation. All cases were operated by the same surgeon. The efficacy, the safety and the patient comfort were evaluated.

Results No statistically significant difference found between the two groups regarding efficacy and complications. Group 2 was superior to group 1 regarding patient satisfaction (p<0.005).

Conclusion 23g surgical equipment offers similar efficacy to the established 20g equipment for macular hole surgery. Though reduces the intraoperative time and patient overall satisfaction is greater.

■ 3415

Face down posturing for macular hole surgery. Is it really required?

TRANOSP

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Purpose Background: In macular hole surgery pars plana vitrectomy and intravitreal gas injection with or without inner limiting membrane peel, is considered the mainstay of treatment. The requirement for face down posturing is generally regarded as part of the traditional postoperative routine. Several mechanisms have been postulated to explain the action of the gas bubble including exertion of a large floatation force on the macula and prevention of the macular hole exposure to vitreous fluid. Recently the need to face down has been challenged since this regime compromises patients' postoperative quality of life and it makes macular hole surgery almost impossible for individuals with mental or physical limitations.

Methods Review of personal data and systematic literature review of studies investigating macular hole surgery with shortened or eliminated face down posturing.

Results There is considerable body of evidence suggesting successful anatomical and functional outcome in patients with shorter duration of posturing or no posturing at all following macular hole surgery. The pros and cons of each technique will be presented in detail.

Conclusion Prone posturing following macular hole surgery provides no functional or anatomic benefit but it is associated with slower progression of cataract. Combined phacovitrectomy without face down positioning may be considered for phakic patients undergoing macular hole surgery.

■ 3416

Sulfur hexafluoride (SF₆) versus perfluoropropane (C₃F₈) gas tamponade for macular hole surgery

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Purpose In Macular Hole surgery gas tamponade is hypothesized to enhance macular hole closure after removal of tangential force. Sulfur hexafluoride (SF₆) was described in the initial report of Macular Hole surgery (MHS). Long lasting gas (such as C₃F₈) may offer more extensive tamponade. To compare outcomes of Macular Hole Surgery using SF₆ gas versus C₃F₈ gas for idiopathic macular hole repair.

Methods A consecutive group of patients undergoing MHS with SF₆ group A (24 eyes of 24 patients) and a consecutive comparison group B with S₃F₈ was used (19 eyes of 19 patients). All patients had PP Vitrectomy, ILM peeling, using Kenacort or Blue and two different gases for internal tamponade.

Results The macular hole closure rate was similar in both groups.

Conclusion Macular Hole surgery using SF₆ gas yields similar results as with C₃F₈ gas and may be a good option.

■ 3421

The initial consultation

SPILEERS W

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Purpose A patient reporting flashing lights, shimmering, positive scotoma, hallucinations, ...needs a full (neuro-)ophthalmological work-up.

Methods The ophthalmological work-up includes visual acuity testing, biomicroscopy of the anterior segment, examination of the fundus. In selected cases, more specific ophthalmic testing such as visual electrophysiology is needed. Sometimes a general work up is indicated.

Conclusion The different neuro-ophthalmological causes of reporting positive phenomena will be introduced.

■ 3422

Retinal causes

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Purpose To describe conditions which affect retinal function and can lead to seeing things which aren't there.

Methods A case presentation format will be used to illustrate different genetically and environmentally determined conditions leading to visual hallucinations. Both clinical and electrophysiological phenotypes as well as genotypes will be discussed.

Results Phenotypes of environmentally and genetically determined retinal diseases leading to visual hallucinations are very different. In some instances, the ophthalmologist can make a real difference when systemic treatment is indicated.

Conclusion Very diverse conditions may give rise to seeing things which aren't there. Clinical examination and electrophysiology allow making a correct diagnosis, with sometimes far reaching implications for therapy.

■ 3423

Visual hallucinations and illusions: neurological considerations

BORRUAT FX

Hôpital Ophthalmique Jules Gonin, Lausanne

Visual hallucinations (seeing what is not present) or illusions (perceiving differently what is actually present) can result from either ophthalmological or neurological disorders. This presentation will be focused on the neurological causes of such visual disorders, with an emphasis on the clinical presentations, anatomical correlations and diagnostic procedures.

■ 3424

Electrophysiology

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Purpose To describe the diagnostic electrophysiological features associated with disorders that may present with positive phenomena.

Methods Standardised electrophysiological testing, performed to incorporate and exceed the Standards of the International Society for Clinical Electrophysiology of Vision.

Results Illustrative cases will be used to demonstrate diagnostic electrophysiological features in a variety of disorders.

Conclusion The objective functional data provided by electrophysiological testing are of considerable importance to the diagnosis and management of these conditions.

■ **3431**
Predicted long-term outcome of corneal transplantation

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Purpose To analyze graft survival and the outcome of the corneal endothelium after corneal transplantation in a single model to predict the long-term prognosis of these grafts.

Methods Cohort study. Data were prospectively recorded and then analyzed retrospectively. Participants: One thousand one hundred and forty-four consecutive eyes of 1,144 patients who underwent corneal transplantation between 1992 and 2006. Interventions: Penetrating keratoplasties and deep anterior lamellar keratoplasties. Main Outcome Measures: Slit lamp examination and wide-field specular microscopy. A joint analysis of endothelial cell loss and time to graft failure was undertaken. From mid-term simultaneous analysis of graft survival and endothelial cell loss, long-term graft survival was predicted.

Results The observed 5- and 10-year graft survival estimates were, respectively, 74% and 64%. The average endothelial cell density (cell loss) was 2,270 cells/mm² preoperatively, 1,058 cells/mm² (-53%) during the 6th post-operative year, and 865 (-61%) cells/mm² during the 10th post-operative year. Overall, the predicted graft survival estimate was 27% at 20 years and 2% at 30 years. Both observed and predicted graft survival were higher in patients with lamellar keratoplasty than in patients with penetrating keratoplasty and normal recipient endothelium and higher in patients with penetrating keratoplasty and normal recipient endothelium than in patients with penetrating keratoplasty and impaired recipient endothelium.

Conclusion For corneal diseases involving the endothelium, penetrating keratoplasty appears to be a good therapeutic approach in elderly patients as the graft lifespan may be similar to the patient life expectancy. Conversely, for younger patients, penetrating keratoplasty is only a mid-term therapeutic approach. For corneal diseases not involving the endothelium, deep anterior lamellar keratoplasty appears to be a promising therapeutic approach with higher long-term expected survival.

■ **3433**
Experimental assessment of endothelial viability of grafts

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 (2) Lab 'Biology, engineering, and Imaging of Corneal Grafts' JE2521, IFR143, Saint-Etienne

Purpose Endothelial quality of penetrating keratoplasty or posterior lamellar grafts (be they manually, mechanically or femto laser dissected) remains of paramount importance for the graft survival in recipients, especially when the recipient endothelial cell peripheral reserve is weak or absent (bullous keratopathy)

Methods 1/Review of the literature on the available methods to measure the endothelial quality. 2/presentation of a new concept: experimental quantification of the actual endothelial cell density of cell deemed viable, thus representing the actual number of cells capable of surviving in the recipient eye. This method uses a live/dead assay coupled with the microscopy analysis of the whole endothelium. A comparative series of graft pre-cut for DMEK versus intact organ cultured paired corneas will illustrate our method

Results For the first time the exact number of viable endothelial cells can be directly measured. The notion of viable endothelial cell density is introduced. Though it cannot be a routine technique but only a laboratory one, the method can be -and perhaps should be- applied for all kind of graft preparation during its experimental development. Lamellar posterior grafts are good candidates. As expected, the viable ECD is lower than the ECD determined with the routine method used in eye banks, even the most precise ones

Conclusion The new notion of viable ECD may participate in our understanding of the dramatic endothelial cell loss ineluctably observed shortly after all types of grafts

■ **3432**
Beyond penetrating keratoplasty: the challenge of endothelial transplantation

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The paper will present a complete review of the technique of endothelial keratoplasty (EK). History as well as basic principles necessary to understand the mechanisms of EK will be introduced to the attendees. In setting the indication to EK, the author will discuss the role of recipient corneal status, type of endothelial disease, concomitant eye disease (i.e. glaucoma), presence of clear lens and other preoperative factors. The different techniques of Descemet stripping automated endothelial keratoplasty (DSAEK) and Descemet membrane endothelial keratoplasty (DMEK) will be presented, pointing out the single steps, which are instrumental in facilitating surgery, while improving the final outcome. In case of multiple intervention, combined versus sequential procedures (e.g. phacoemulsification, IOL surgery, vitrectomy, etc.) will be discussed. The authors will highlight advantages and disadvantages of different surgical approaches. Possible implications for eye banking will also be addressed, with particular emphasis on preparation and storage of tissue for DSAEK and DMEK. Slides and videos of case studies will illustrate the most common complications (i.e. graft detachment, dislocation, failure, rejection etc.) as well as the appropriate solutions.

■ **3434**
Endothelial grafts with manually dissected endothelio-Descemet. Eye bank and surgical point of view

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Purpose to describe a manual technique for the preparation of thin endothelial grafts or DMEK and to report clinical results in patients with Fuchs dystrophy.

Methods We report here a series of 92 patients with Fuchs dystrophy undergoing endothelial keratoplasty. A manual dissection is performed on an artificial chamber to obtain posterior graft. After completing the learning curve, it is possible to select the depth of dissection: from pure descemet membrane isolation, to very thin posterior graft.

Results Visual acuity is significantly higher after DMEK (0.7) than after thin posterior grafts (0.58), 6 months after surgery. However, endothelial density is much lower with 1190 cel/mm² after DMEK compared to thin posterior grafts which have 1870 cell/mm² 6 months after grafting.

Conclusion Manual dissection is more difficult to perform than automated techniques but allow preparation of much thinner grafts. Among these, pure descemet membrane grafts have the best visual rehabilitation but less endothelial cells due to surgical manipulations.

■ 3435

Insights into regulation of the human corneal endothelial cell cycle

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(2) *Dept. of Ophthalmology, Harvard Medical School, Boston*

Purpose Human corneal endothelial cells (HCEC) exhibit both an age- and position-related decrease in proliferative capacity. HCEC are inhibited in G1-phase of the cell cycle, but this not caused by critically short telomeres. The current studies explored whether oxidative stress and oxidative DNA damage could be responsible for this decreased proliferative capacity.

Methods Human donor corneas were divided into 2 age-groups: Young=<30yo; Older=>50yo. Ex vivo corneas were used for some studies and, for others, HCEC were cultured according to published protocols. An ELISA assay quantified 8-OHdG, a marker of oxidative DNA damage. ICC was used to localize 8-OHdG. Treatment of HCEC from young donors with H₂O₂ was used to test the effect of oxidative stress on nuclear DNA damage and relative proliferative capacity.

Results Significant oxidative DNA damage was present in HCEC from older donors, particularly in central endothelium. 8-OHdG staining was present in mitochondria in central and peripheral HCEC from young donors. In HCEC from older donors, intense 8-OHdG staining was found in nuclei of central cells, indicating nuclear oxidative DNA damage. Age-related differences in nuclear 8-OHdG staining were also found in cultured HCEC. Treatment of HCEC from young donors with H₂O₂ caused a dose-dependent increase in nuclear 8-OHdG staining and a dose-dependent decrease in proliferative capacity. Growth curves from H₂O₂-treated young HCEC closely resembled those of untreated HCEC from older donors.

Conclusion The age- and position-related decrease in proliferative capacity observed in HCEC is due to increased nuclear oxidative DNA damage, possibly caused by chronic light exposure and high metabolic activity.

■ 3436

Developing gene therapies for corneal endothelial disorders

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Techniques of gene transfer to corneal cells have been in development for at least 15 years. Studies on feasibility of gene transfer and kinetics of transgene expression have been undertaken ex vivo and in vivo using physical, viral vector and non-viral vector techniques of gene transfer. Functional gene transfer research has been developed for applications such as anti-angiogenesis, modulation of stromal wound healing or endothelial cell cycle control and prevention of allotransplant rejection. The absence of such application in inherited disorders of corneal endothelium points to absence of in vivo models for study and the availability of surgical treatment which, in many patients, is effective. Opportunities in the field of gene-based approaches to disorders of corneal endothelium lie in newer designs of low-immunogenicity vectors, longer duration of transgene expression and application in eye banking. Experimental approaches which in essence use cDNA as an alternative to protein or drug treatment will be discussed. Supported by NIHR Biomedical Research Centre in Ophthalmology, Moorfields Eye Hospital & UCL Institute of Ophthalmology, London, United Kingdom

■ 3441

Oxidative stress from in vivo dual waveband exposure to ultraviolet radiation

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Purpose The purpose of the current project was to elucidate if the effect of exposures to two wavebands is directly proportional to the sum of the biologically efficient dose for each of the two wavebands.

Methods Altogether 40 albino Sprague-Dawley rats, 6 weeks old (150 g), were exposed unilaterally in vivo to UVR. Half the group was exposed to 8 kJ/m² biologically efficient dose (= absolute dose) of UVR centered at 300 nm. The other 20 rats were exposed, first to 4 kJ/m² biologically efficient dose (= absolute dose) of UVR centered at 300 nm, and then to 4 kJ/m² biologically efficient dose (= 42 kJ/m² absolute dose) centered at 310 nm. All animals were sacrificed at 1 week after exposure and intensity of forward light scattering was measured in both lenses.

Results The UVR 300+310 nm group expressed slightly more light scattering than the UVR 300 nm only group as indicated by a 95 confidence interval for the difference approximating the degrees of freedom due to different variances (CI:D(0.95) = -8.69 +/- 6.92 x10⁻² tEDC, df = 32.7).

Conclusion Although, the outcome indicated that there is a difference between the two groups, the difference is very small in relation to spectral sensitivity. It is therefore concluded that it is a valid approximation to assume spectral additivity of biologically efficient doses when estimating effects of broadband exposures.

■ 3443

The role of mitochondrial glutaredoxin-2 in protecting cells against oxidative stress

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Purpose Glutaredoxin 2 (Grx2) is an isozyme of glutaredoxin (Grx1) present in the mitochondria. Similar to Grx1, it has dethiolase activity but the general function is largely unknown. In this study, we investigate the potential anti-apoptotic function of Grx2 through its ability to protect complex I enzyme in the mitochondrial electron transport system.

Methods Grx2 plasmid or Grx2 siRNA was transfected in human lens epithelial B3 cells (HLE-B3) to generate Grx2 overexpressed (OE) or Grx2 knockdown (KD) cell line. Cells with empty vector were used as the control. All three cell lines were incubated with and without H₂O₂ (0.2 mM) for 24 hrs before examining for cell viability (WST8 colorimetric method). Apoptosis was measured by flow cytometry, and the protein levels of Bax, Bcl-2, cytochrome C and caspase 3 activation by Western blot analysis using specific antibodies. In some experiments, rotenone (0.5 mM), a specific inhibitor of complex I, was used together with H₂O₂ to evaluate complex I activity protected by Grx2.

Results Grx2 OE protected HLE-B3 cells from H₂O₂-induced cell viability loss and apoptosis. These cells also exhibited high level of Bcl-2 and inhibited cytochrome C release and caspase 3 activation induced by H₂O₂. In addition, Grx2 OE also protected mitochondria from H₂O₂-induced loss in complex I activity. In contrast, Grx2 KD enhanced H₂O₂-induced cellular damage and complex I inactivation.

Conclusion Grx2 can protect HLEB3 cells against H₂O₂ induced apoptosis. The mechanism of this protection is likely associated with its ability to protect complex I in the electron transport chain and preserve the mitochondrial membrane integrity.

■ 3442

Oxidative effects on the glutathione enzyme systems

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Purpose Lens ageing is associated with oxidative processes, with photo-oxidation as one of the stressors. Cataract has traditionally been viewed as a one-way progressive event, but in vivo and in vitro animal studies on ultraviolet radiation-induced cataract during the last years show repair capacity in the lens. Little is known about what these repair systems are and how they might be influenced by pharmaceuticals. The glutathione redox enzymes have critical antioxidative functions and are a potential target for pharmaceutical intervention.

Methods Primary cultures of lens epithelial cells were obtained from mouse and human pediatric lens capsules. The cells were stressed with varying concentrations of hydrogen peroxide. The short-term activity pattern of primary glutathione-reducing enzymes and other glutathione-associated enzymes were determined.

Results The main picture was a concentration-dependent decrease in enzyme activity within 15 minutes, with subsequent recovery within one hour.

Conclusion The time frame for enzyme inhibition is co-occurring with that of maximally efficient exposure time in ultraviolet radiation-induced cataract.

■ 3444

Enhanced age-related and diabetes-induced cataract in mice lacking CuZn-superoxide dismutase

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Purpose In the lens, light and oxygen generate harmful reactive oxygen species (ROS) that may cause age-related cataract. In diabetes mellitus, increased glucose levels may contribute to increased generation of ROS which may accelerate cataract formation. We have here explored the role of the antioxidant enzyme copper-zinc superoxide dismutase (SOD1) in the protection against age-related and diabetes-induced cataract development.

Methods Cataract formation in relation to the oxidative status of the lens was evaluated in streptozotocin-induced diabetic as well as in non-diabetic SOD1 null and wild-type mice. Also, the spontaneous age-related cataract development was followed in both genotypes.

Results Cataract was seen in the SOD1 null and the wild-type mice after 8 weeks of diabetes, although the SOD1 null mice showed a more pronounced cataract formation than the wild-type mice in relation to the level of hyperglycaemia. As cataract formation was accentuated the lenses showed diminishing levels of glutathione but increasing amounts of protein carbonyls, suggesting a reduced lens anti-oxidative capacity as well as increased lens protein oxidation. Spontaneous incipient age-related cataract was seen in the 1-year old SOD1 null mice whereas the wild-type mice showed equivalent changes at 2 years of age.

Conclusion The results presented here show that SOD1 null mice are more prone to develop diabetes-induced and age-related cataract than wild-type mice. The findings thus further endorse the importance of oxidative stress as a contributor to cataract development and indicate that superoxide radicals may be damaging to the lens. We therefore conclude that the antioxidant enzyme SOD1 is important for the protection against cataract.

■ 3445

Orally active multi-functional antioxidants for the treatment of cataracts and AMD

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Age-related cataract and neurodegeneration has been linked to oxidative stress and increased lenticular levels of Fe and Cu, which can contribute to ROS generated through the Fenton Reaction. Since both antioxidants and chelating agents have both been reported to reduce experimental cataracts, we have synthesized a new class of antioxidants (JHX-4 and its dimethoxy analog JHX-8) containing a novel 2-amino-4-hydroxypyrimidine ring system that selectively chelates Fe and Cu. In vitro studies in human lens epithelial cells and retinal pigment epithelial cells demonstrate that these compounds can reduce ROS generated by H₂O₂, endoplasmic reticulum (ER) stress, or Fenton's reaction. In vivo studies demonstrate that these compounds accumulate in the lens and retina after oral administration. In Long-Evans rats receiving whole head irradiation, these compounds delayed cataract formation proportional to the tissue levels of drug achieved. Compared to untreated rats, treatment with JHX-4 and -8 delayed the formation of PSC punctuate opacities in 50% of animals by 53 and 58 days, respectively and lens PSC opacities by 38 and 47 days, respectively. In vivo neuroprotection studies are currently in progress. Supported by NIH EY016460-01.

■ 3446

The ubiquitin proteasome pathway - Repair or degradation of damaged proteins

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Purpose Accumulation of methylglyoxal (MGO), a highly reactive side-product of glycolysis can modify proteins. Increased levels of MGO in cells have been implicated in diabetic vascular complications. In physiological conditions, proteolytic systems and chaperones together ensure maintenance of protein quality control. We hypothesize that MGO impairs the function of UPS and on molecular chaperones.

Methods Rats with moderate type 2 diabetes (GK) and retinal epithelium pigment cell line were used. Protein oxidation was assessed by formation of carbonyl groups. Production of intracellular ROS was assessed in frozen sections of diabetic retinas by DHE incorporation. 20S proteasome activities were assessed by fluorogenic peptides. Ubiquitin (Ub) conjugation activity was determined by the ability of retinal extracts to conjugate 125I-Ub to endogenous substrates. Ub conjugates, Hsp90, Hsc70, Hsp40 and CHIP levels were assessed by WB. Cell viability was determined by MTT while proliferation was assessed by BrdU-incorporation.

Results Data show that accumulation of endogenous Ub conjugates in the presence of MGO is associated with an increased ability of retinal extracts to conjugate 125I-Ub to endogenous substrates. Moreover, MGO significantly decreases the 20S proteasome activity. Data further show that MGO decreases the levels of the molecular chaperones Hsp90 and Hsc70 and promotes aggregation of Hsp40 and CHIP. Moreover, these aggregates revealed immunoreactivity against Ub. Consistently, these effects are associated with increased cell protein oxidation, decreased cell proliferation and viability.

Conclusion In diabetes, accumulation of MGO may impair the UPP and the protein quality control, leading to accumulation of obsolete proteins and cell injury.

■ 3451

We're still under pressure: postoperative hypertony

STALMANS I
Leuven

Purpose Postoperative hypertony is a frequent complication after trabeculectomy. This problem may occur from the early, to the late stages, and may be an acute or chronic situation. The aim of this lecture is to review the various causes with their clinical presentation and therapeutic strategy.

Methods An overview will be provided on the different mechanisms that may cause hypertony after trabeculectomy, ranging from tight flap sutures or blocking of the constructed channel by various substances, over encapsulation and failing bleb to steroid response. For each of these situations, the clinical presentation and differential diagnosis will be discussed. Finally, the therapeutic options will be reviewed. Photographic and video material will be used to illustrate the various clinical presentations and therapeutic interventions.

Conclusion This lecture will provide the audience with a practically oriented overview of the clinical management of hypertony after trabeculectomy.

■ 3452

Help, I've been too successful: postoperative hypotony

ZEYEN T
Leuven

Purpose To give an overview of the etiology and treatment of hypotony post-trabeculectomy.

Methods Postoperative hypotony is most commonly caused by either overfiltration or bleb leakage. This condition can lead to a flat anterior chamber, hypotony maculopathy, choroidal detachment, and cataract.

Results Possible strategies to decrease overfiltration and heal bleb leaks will be highlighted. We will review when and how to refill the anterior chamber, and discuss the proper timing and technique to treat choroidal detachments. Finally, the indications, pros and cons of cataract surgery after trabeculectomy will be discussed.

Conclusion This presentation will give guidance on how to diagnose and treat hypotony after trabeculectomy.

■ 3453

Cataract, loss of visual acuity, infection after trabeculectomy

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Purpose Trabeculectomy is the most popular surgery for glaucoma, however some complications may impair the success of the procedure. Moreover without impacting the overall efficacy of trabeculectomy, the quality of life of the patients could be affected in a certain number of cases.

Methods In this session we will give some tips to prevent and to treat these complications.

Results Cataract is frequent after trabeculectomy and is mainly related to postoperative hypotony. In most recent clinical studies cataract has been shown to occur in half of the eyes 3 years after trabeculectomy. This has led H Jampel to write a provocative editorial; Trabeculectomy: more effective at causing cataract surgery than lowering intraocular pressure (Ophthalmology 2009;116:173-174). In advanced cases, severe sight-threatening complications such as the wipe-out syndrome, even uncommon may definitely lead the patient to blindness. The two more frequent presentations of infection after trabeculectomy are blebitis and endophthalmitis. Both can occur several years after trabeculectomy and Streptococci which are frequently found are devastating strains. Antimetabolites greatly increase the prevalence of endophthalmitis after trabeculectomy. Other less severe complications such as bleb dysesthesia may alter the quality of life of the patients.

Conclusion The information of the patients and the quality of the follow-up are of paramount importance when a trabeculectomy is considered.

■ 3461

Non-viral strategies of intra-ocular gene delivery

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Purpose Systemic anti TNF strategies are efficient to treat intraocular inflammation but require repeated injections and are associated to severe systemic side effects. Our aim was to develop a non viral gene transfer method to produce locally anti-inflammatory proteins in a sustained and minimally invasive manner in the ocular media. For this purpose, we have transformed the ciliary muscle into a bioreactor, using an electrically assisted gene transfer technique.

Methods Electrotransfer (ET) of plasmids, encoding for different variants of TNF alpha soluble receptors, was performed in the ciliary muscle cells. Using optimized conditions, soluble receptors were dosed in the ocular media up to 8 months after a single treatment. The technique has been applied in two models of intraocular inflammation: Endotoxin-Induced Uveitis (EIU) and auto immune experimental uveitis (EAU) in rats.

Results When performed 8 days or 3 months before the LPS challenge, ET significantly reduced both clinical and histological signs of EIU. Particularly, iNOS, IL6 and TNF were down regulated while IL10 was upregulated. Importantly, systemic TNF alpha was not decreased demonstrating a local effect of the treatment. In EAU, ET significantly delayed the onset of EAU and decreased its severity. Similarly, a switch towards a Th2 cytokines profile was observed in the ocular media without any effect on systemic TNF alpha.

Conclusion - ET is a safe and efficient non viral method to produce locally TNF alpha soluble receptors. - Local anti TNF allows for a local intraocular immunomodulation, without affecting systemic TNF. ET could therefore be used to reduce systemic side effects of anti TNF and prevent repeated injections.

■ 3463

Regulation of transgene expression

KOCH P (1, 2)

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Purpose Regulation of the transgene expression in the targeted cells is of course of major importance when using gene therapy. Actually, we have a huge range of possibilities to regulate gene expression.

Methods There are two main classes of promoters: constitutive and inducible promoters. Amongst constitutive promoters, we have two sub-forms: non-tissue and tissue specific promoters. The last allows us to better target the tissue or cells in which we want to express our gene of interest. On the other hand, inducible promoters have been widely developed recently and allow us to obtain a regulated expression, depending on different factors. Very recently, disease specific inducible promoters emerged for a more precise regulation.

Results We will together examine more precisely the different possibilities offered by gene regulation in Gene Therapy. Thereafter, we will more specifically describe usable promoters in ocular inflammation. Finally we will examine the effects of some inflammatory, disease specific, promoters.

Conclusion Regulation of transgene expression is one of the fundamentals of efficient gene transfer. Recent developments actually allow us to play within the targeted cell(s) to obtain an expression in specific conditions.

■ 3462

Immune responses to gene therapy vectors in the context of corneal transplantation

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Purpose The genetic engineering of grafts or cells prior to transplantation is an attractive approach to protect the graft from allogeneic rejection. Virus vector-based gene therapy is a promising method for successful ex-vivo gene transfer however, the induction of an immune response against gene-modified tissues raises concern.

Methods Different virus families (Adenovirus, Retrovirus, Adeno-associated virus, Herpesvirus) have been studied as gene therapy vehicles for the delivery of therapeutic molecules. Moreover, different serotypes or envelope proteins have been used to modulate transduction efficiencies of target cells or to evade pre-existing immunity.

Results Here we review gene therapeutic applications using viral vectors in the context of cornea transplantation. Both local and systemic expression of immunomodulatory molecules have led to the prevention of corneal graft rejection. However, different results have been obtained with regard to the induction of immune responses after local or systemic expression of the gene therapy vector. Not surprisingly over-expression of anti-inflammatory molecules not only modulated allograft rejection but also influenced the immune response against the viral vector and virally transduced cells.

Conclusion Recent clinical trials indicate that the application of viral vectors in ophthalmology is promising however, the generation of immune responses against the viral vector or virally transduced cells are still a serious obstacle for a broader application of gene therapy. Supported by Deutsche Forschungsgemeinschaft (DFG PI 150/14-1 and Ri 764/10-1) and Science Foundation of Ireland (SFI 06/RFP/BIC056 and SFI 07/IN.1/B925)

■ 3464

Gene therapy for corneal transplantation

PLEYER U

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ABSTRACT NOT PROVIDED

■ 3465

Gene therapy: can we prevent/modulate apoptosis in EC?

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Purpose Regardless of the inciting cause, CEC loss is a common denominator of corneal graft failure. CEC loss during storage results in significant loss of suitable tissue for grafting. CEC loss after transplantation is a major cause of graft failure. The purpose of this study is to investigate the role of apoptosis in CEC in order to prevent CEC loss during storage.

Methods Gene transfer of Lenti-Bcl-xL or -p35 was accomplished in human donor corneas, primary cultured CEC and an immortalized CEC line and compared to untreated controls. Cell death (apoptosis) was induced by Actinomycin or Etoposide (external vs. internal apoptotic pathway, respectively). In addition, CEC loss during preservation was studied both during Optisol GS (4C) and organ culture storage (37C, Biochrome Medium I). Both storage media were diluted with PBS to promote cell loss. CEC were enumerated, apoptosis was detected by TUNEL staining and confocal microscopy.

Results The percentage of TUNEL-positive CEC provoked by the apoptotic inducers was significantly reduced relative to controls. Transfected corneas preserved an almost intact endothelial monolayer while controls nearly entirely lost vital CEC. During long-term storage experiments at 4C and at 37C, CEC counts in corneas expressing anti-apoptotic genes remained significantly higher compared to the controls.

Conclusion Protection of CEC by anti-apoptotic genes appears to be an effective method to reduce CEC loss during storage. The application of this technique could increase the amount of high quality grafts in eye banking and further reduce graft failure following corneal transplantation, and is of specific interest as to pre-cut corneas and DSAEK procedures.

■ 3466

Gene therapy for posterior uveitis

DICK AD

Department of Cellular and Molecular Medicine, Bristol

Purpose To investigate the role of gene therapy incorporating release of immunomodulatory cytokines in animal models of intraocular inflammation

Methods By inoculating with either AAV or lente viruses incorporating genes for IL-1RA or IL-10 into either the anterior chamber or subretinally we observed the ability to suppress either endotoxin induced uveitis (EIU) or experimental autoimmune uveoretinitis (EAU).

Results Anterior chamber inoculation with lente-IL-10 or IL-1RA successfully suppresses inflammation and protein exudation into the eye during the course of EIU. Subretinal injection of AAV-IL-10 suppresses EAU. The extent of local macrophage activation is also suppressed as there is marked reduction in nitrotyrosine expression within the retina.

Conclusion Gene therapy with immunomodulatory cytokines offers a potential to suppress active inflammatory processes within the retina. Mechanisms will be discussed in the talk in relation to macrophage activation and restoring myeloid cell (microglial) homeostasis within the retina.

■ 4111

Non surgical approach in diabetic macular edema: the future?

CHIQUET C

Department of Ophthalmology - University Hospital, Grenoble

Purpose To present the different non surgical therapeutical options of diabetic macular edema

Methods The pathogenesis of diabetic macular edema is multifactorial. Hyperglycemia and poor systemic factor balance are major risk factors. Laser treatments and antiangiogenic treatments represent the main non surgical options to treat macular edema.

Results Focal macular edema remains the best indication of laser treatment. Laser remains also the standard of care of diffuse macular edema but some edemas remain resistant. Several therapeutic options have been proposed: Steroid intravitreal injection and antiVEGF therapy (either PKC inhibitors, VEGF aptamers or VEGF antibodies) represent the future alternative treatments as well as their potential combination.

Conclusion Laser remains the main treatment of diabetic macular edema. However, steroids and antiangiogenic agents either isolated or combined represent the main alternative treatment for non responding diffuse macular edema.

■ 4112

Which place for surgery for macular edema due to diabetic retinopathy?

JONAS JB

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Purpose To present treatment options for macular edema

Methods The various types of macular edema will briefly be discussed and available and potentially future treatment strategies will be presented.

Results The results of the current multicenter trials as well as the findings of previous studies using different medical agents for the treatment of diabetic macular edema will be compared.

Conclusion It may still be unclear which treatment strategy appears to be the best for which type of diabetic macular edema.

Commercial interest

■ 4113

Is there still a place for vitrectomy in the treatment of macular edema due to venous occlusion?

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Purpose Persistent macular edema (ME) is the main cause of poor visual outcome in either non-ischemic BRVO or CRVO. Among multiples treatment approaches, vitreoretinal surgery with the goal to achieve the recanalisation of the occluded vessels and/or the resolution of ME, were proposed.

Methods Vitrectomy with peeling of the posterior hyaloid and/or the internal limiting membrane, associated to intravitreal (IVT) triamcinolone, neurotomy, sheathotomy, intravascular rtPA injection were studied in numerous nonrandomized cases series.

Results Pars plana vitrectomy has been shown to reduce macular oedema and restore the normal foveal contour without significant change in best corrected visual acuity. In contrast, visual improvement occurs after vitrectomy for vitreous haemorrhage, epiretinal membrane formation and retinal detachment complicating BRVO. Evidence to date does not support any therapeutic benefit from radial optic neurotomy, optic nerve decompression, arteriovenous crossing sheathotomy or intravascular rtPA. Vitrectomy combined with IVT triamcinolone, induces a ME decrease rapidly and durably, without any improvement in visual acuity.

Conclusion Vitrectomy with IVT triamcinolone seems to have a more durable effect than IVT triamcinolone alone. Vitrectomy, A-V sheathotomy combined with intravenous t-PA may offer benefits in BRVO. Despite uncertainty and open questions, surgical interventions are likely to be a therapeutic option for RVO in the future. Randomized and controlled studies are needed to confirm these results and to compare them to the natural course of the disease.

■ 4114

Macular edema in epiretinal membrane and vitreomacular traction

CREUZOT C

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Purpose Macular edema occurring during macular diseases is a frequent situation. The purpose of this course is to highlight the clinical signs and the different treatments which can be used to treat macular edema occurring during epiretinal membrane and vitreomacular traction syndrome.

Methods Macular edema is frequently associated with epiretinal membranes and vitreomacular traction. Up to now, no specific preoperative macular edema phenotype can predict the postoperative recovery. Different methods were proposed to improve functional results: ILM peeling, intravitreal steroid injection, intravitreal antiangiogenic injections...

Results The widespread use of transconjunctival vitrectomy, the combination of cataract and macular surgeries will probably change the indication of macular surgery leading to sooner surgical indications for better recovery. By contrast, vitreomacular traction often leads to a rapidly progressive visual loss. The analysis should distinguish diffuse diabetic macular edema which remains the only validated surgical indication of macular edema in diabetes and the vitreomacular traction without diabetes. This latter needs a rapid surgical management as macular edema is often severe.

Conclusion Macular edema is a frequent situation associated to macular disease. It can compromise the outcomes after macular surgery. Attempts to define the best moment to consider surgery should improve the functional results.

■ 4115

Macular edema and uveitis: may we find a place for surgery

DESMET M

ABSTRACT NOT PROVIDED

■ 4121

History of and necessity for KPros

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The history of keratoprotheses goes back over 200 years. There was a resurgence in interest in the second half of the twentieth century as it was recognised that keratoplasty could not solve all types of corneal blindness. Many devices have been described but few have survived. Corneal transplantation is complicated by graft rejection and astigmatism. There is also a problem with adequate supply, and there is a risk of transmission of infection. There is a desire for an artificial cornea which surpasses cadaveric transplantation. There is much ongoing work, but the majority of clinical work on keratoprotheses are for corneal blindness not amenable to cadaveric grafts. These can be separated into two main groups. The wet blinking eye which have had multiple graft failures, and the dry eye with a keratinised ocular surface which may also have a deficiency in lid cover. The approaches to these are quite different.

■ 4123

Boston keratoprosthesis

DOHLMAN CH

Cornea, Boston

Purpose To outline the present designs, recent outcomes and acceptance of the Boston devices. In addition, several areas of new developments will be briefly discussed.

Methods The Boston KPros implanted in Boston from 1990 to present provide the clinical basis for recent studies on materials, optics, drug delivery, intraocular pressure transducers, complications such as infections and retinal detachment, cost-effectiveness, outcome in the developing world, etc. The details are presented in accompanying posters.

Results More than 3000 Boston Keratoprotheses have so far been implanted worldwide. Recent outcome studies that have been published from centers outside Boston are particularly suitable for meta-analysis. For outcome of recent research in Boston, see posters.

Conclusion The Boston Keratoprotheses have shown clinical value but there is still a substantial gap to fill before having arrived at a simple, safe and inexpensive keratoprosthesis procedure for all corneal blindness, in all parts of the world.

■ 4122

The first keratoprosthesis implantation in 1955

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Purpose to describe the Barcelona experience with the different types of keratoprosthesis for end-stage cicatricial corneal disease.

Methods We present 6 cases showing various types of keratoprosthesis which we have used at the Barraquer Eye Center from the 1950's up to the present.

Results We describe the first keratoprosthesis ever implanted in Spain, on a case of severe chemical burn on a young lady. We implanted a Dorzee acrylic keratoprosthesis in 1955. 5 years later she had extrusion of the prosthesis and superior retinal detachment. The second case describes the Dorzee-Barraquer-Cardona acrylic implant implanted in 1958 on a patient with end-stage glaucoma. Patient had good anatomical retention until his death in 1970. The third case describes the use of the Cardona keratoprosthesis in 1960. Patient had good anatomical and functional results for 8 years until suffering from an acute endophthalmitis. The fourth case demonstrates the expulsion of a Teflon-supported keratoprosthesis designed by Girard. The fifth case describes the first implantation of an osteo-odontokeratoprosthesis designed by Strampelli on a blast injury showing good results for 10 years. The last case shows our experience on the Boston keratoprosthesis which we started using in 2006. Finally, we present our technique of the osteo-odontokeratoprosthesis and a summary of our clinical results from 1970's to the present.

Conclusion Our clinical experience for more than 50 years on the use of keratoprosthesis shows that while the surgical technique, design and post-operative treatment of both biological and non-biological keratoprosthesis have improved thru the years, the anatomical and functional success remains a challenge for the KPro surgeon.

■ 4124

Boston type I in pediatric patients

AQUAVELLA J, CHAK G

Rochester

Purpose To present a retrospective review of keratoprosthetic implantation and retention in patients with congenital corneal opacities.

Methods Pediatric patients younger than seven years old, the average age of permanent visual loss from understimulation of the visual cortex, were selected from a single center Boston Type I keratoprosthesis database and categorized by 1) primary diagnosis, 2) short-term visual outcome, and 3) post-operative complications.

Results Seventeen patients, with an age range of 41 days up to 6 years, were selected from a database of over one hundred and forty patients. Six had a primary diagnosis of sclerocornea and eleven had Peter's anomaly. Visual outcome after one year improved in fourteen of the patients, with patients who previously could not detect light to subsequently being able to fixate and follow or even read allen cards. The remaining three patients showed no improvement in visual acuity but also no worsening from their baseline condition. In terms of post-operative complications of the optic, two had retroprosthetic membrane formation, and another patient required replacement of the keratoprosthesis due to phthisis and optic melting. From a retinal standpoint, four patients had retinal detachments. There were no cases of choroidal hemorrhaging or hypotony in these patients.

Conclusion Based on visual outcome, the Boston Type I keratoprosthesis is a safe and effective procedure for patients with congenital corneal opacities. With great retention, the artificial cornea is a viable option for prevention of amblyopia. Due to comorbidities such as congenital cataracts, congenital glaucoma, and retinal detachments, it is crucial to have glaucoma and vitreo-retinal surgeons on hand when managing and implanting keratoprosthesis in a pediatric population.

■ 4125

Design, surgical technique and complications MOOKP

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Purpose To describe design, surgical technique of the modified osteo-odonto-keratoprosthesis (MOOKP), originated from Strampelli's OOKP, with the modifications and the innovations made by Falcinelli, between these those ones of the last years to the first stage of the procedure, never published.

Methods First stage: abstraction of the mono-rooted tooth with surrounding root and bone. Preparation of the lamina to which, in the dentine central part, the PMMA optic cylinder is glued. Burying of the lamina for 3 months (subcutaneous pocket). New modification: opening of the A.C., complete removal of the iris, lens cryo-extraction, anterior vitrectomy. Intermediate stage: after 1 month covering of the eye surface by buccal mucosa. Final stage: after 3 months implant of the lamina on the eye with insertion in A.C. of the cylinder after corneal trephining, covering with buccal mucosa previously detached.

Results MOOKP COMPLICATIONS-

1. Intraoperative, easy to be cured.
2. After the 1st stage and intermediate stage: all easily treatable.
3. After the prosthesis implant, more severe:
 - a. Prosthesis complications which affect mainly the mucosa, easy to be cured, or the lamina and the cylinder, more rare, difficult to be cured.
 - b. Eye complications: retinal detachment, not frequent and easily cured. Retroprosthetic membranes, very rare. Endophthalmitis, very difficult to treat, fortunately rare.

Conclusion Biological properties Strampelli's osteo-odonto lamina (OOL) with Falcinelli's surgical modifications make of MOOKP a KPro with best results, theoretically without duration limits in the less and in the very severe cases of corneal and anterior ocular surface alterations like the last stage of dry eye.

■ 4127

AlphaCor, Pintucci, Supradescemeti

STOIBER J, HILLE K

Miami

ABSTRACT NOT PROVIDED

■ 4126

Barcelona OOKP and tibial KPro

TEMPRANO J

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We are using the second technique of the Strampelli OOKP since 1971. Preferably we use a tooth from the superior mandible to prepare the haptic part of the keratoprosthesis. The optic cylinder of 9 mm length, 0.4 mm diameter in the wider part and 3.5 mm in the narrow part is introduced into the prosthesis through the central opening previously prepared for this.

In 1988, trying to find a solution for patients without teeth, we decided to use another heterologous autotransplant, from the tibia. We obtained from the upper part of the medial area of the tibia a disk, 10 mm in diameter and 3 mm thick, and we performed in the center of the same an opening through which we introduced the optic cylinder similar to the one used in OOKP and fixed it to the bone.

The technique is similar for both techniques of keratoprosthesis, excepting the shaping of the support.

The first stage consists in cleaning the anterior portion of the eye, up to the muscle insertions and performing a superficial keratectomy to eliminate the epithelium. Subsequently we obtain from the lower lip of the same patient an oval fragment with a surface of 3 x 2 cm and total thickness including mucous and submucous tissue. This is sutured to the muscle insertions to cover the entire anterior pole of the eye. It is expected to revitalize the surface and will serve later to cover the keratoprosthesis.

The second stage consists in preparing the keratoprosthesis and once it has been completed it is introduced into a palpebral pocket in the inferior lid, so the prosthesis will be covered by soft newly formed tissue which will later facilitate fixation with sutures. The prosthesis is left "in situ" for three months to make sure that it develops neither infection nor immune reaction.

The third stage is performed after three months. The mucosa is separated all over the cornea and an opening of 4.5 mm is performed in the center of the cornea. Previously the prosthesis has been taken out of the palpebral pocket, the wider portion of the optic cylinder is introduced in the anterior chamber, leaving the haptic part on the cornea anteriorly, which means we have a mesoprosthesis, which is then sutured to the eye and covered with the buccal mucosa.

Complications: During the first three months necrosis of the mucosa may develop, which means that a new mucosal graft must be applied or the third stage must be carried out as a transpalpebral procedure. Infection or resorption of the bone support of the prosthesis may also occur, in which case a new prosthesis must be constructed.

Late complications may include by order of frequency: Glaucoma, vitritis, retinal detachment, incipient extrusion of the prosthesis with intraocular infection and, finally, endophthalmitis with total expulsion of the prosthesis and phthisis bulbi.

■ 4131

Gene transfer of disease regulated promoters during experimental autoimmune uveitis

ELMALEH V
Brussels

Purpose Adeno-associated virus (AAV) vectors have been successfully used to transfer immunosuppressive genes into the retina to prevent experimental uveitis development. Transgene expression is classically regulated by constitutive or tetracycline inducible promoters. It might be more advantageous that the control of transgene expression depends on the pathological process itself. Inflammation activates transcription factors acting on promoters containing short responsive sequences, responding, for example to nuclear factor kappa B (NFκB-RE). These responsive elements can be used to generate disease regulated promoters.

Methods An AAV vector with the GFP gene under the control of a NF-κB-RE containing promoter will be injected subretinally in C57Bl6 mice. Autoimmune uveitis will be induced by adoptive transfer of IRBP specific lymphocytes. Animals will be sacrificed at different time points. GFP expression will be analysed by immunofluorescence. VCAM1, MHC II and CD45 will be analysed by immunofluorescence and used to monitor the level of retinal inflammation.

Results One week after disease induction, GFP expression was found in eyes injected with this new vector. Milder GFP expression was also found in mice who did not receive adoptive transfer. This background was increased a 14.

Conclusion Our preliminary results suggest that disease driven GFP expression can be obtained by the use of AAV vectors containing disease regulated promoters. We still need some more times to improve our model. In the future, we plan to replace the GFP gene by an immunosuppressive gene and test if the system can be used to treat experimental uveitis.

■ 4133

Investigating the influence of wavelength, light intensity and macular pigmentation on retinal straylight

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Purpose To investigate the influence of wavelength, light intensity and macular pigmentation on retinal straylight. This will be tested in both phakic and pseudophakic eyes by means of color filters, as well as by comparing postoperative straylight results of eyes implanted with either clear or blue-blocking IOLs. It has also been suggested in the literature that the yellow macular pigment reduces the effects of the short wavelength components of retinal straylight.

Methods In this prospective study the straylight is measured on two groups of pseudophakic subjects: one group of 25 subjects implanted with a clear IOL (Alcon AcrySof SA60AT) and another group of 25 subjects implanted with a yellow IOL (AcrySof Natural SN60AT) using white light as well as with blue, green and red filters. These measurements are repeated on a group of age matched 25 phakic subjects. All retinal straylight measurements will be performed using the Oculus C-Quant straylight meter. In the normal and clear IOL subjects the macular pigment is measured as well using the Tinsley M|Pod device.

Results The first, preliminary results demonstrate that in healthy eyes the retinal straylight measured by the Oculus C-Quant depends on the wavelength of the stimulus light. Measurements performed with blue light were lower than those performed in green or red light.

Conclusion Stimulus light wavelength appears to have an influence on straylight measurements. Further study on a larger population is required to confirm this trend and to study how IOL color may influence this result in pseudophakic eyes. The possible influence of macular pigment on retinal straylight will be studied simultaneously.

■ 4132

Evaluation of cerebrospinal fluid pressure in patients with Alzheimer's disease as a possible cause of glaucoma

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Purpose To investigate whether cerebrospinal fluid (CSF) pressure and trans-lamina cribrosa pressure gradient play a role in the pathogenesis of glaucoma. Our hypothesis is that a low cerebrospinal fluid (CSF) pressure may be correlated with the presence of glaucoma. The first objective is to investigate whether the CSF pressure in Alzheimer's disease (AD) patients with glaucoma is lower than in AD patients without glaucoma. The second goal is to evaluate an animal model with AD for the incidence and prevalence of glaucoma. If glaucoma is present histopathological analysis will be performed on retina and optic nerve, to search for Alzheimer-type changes.

Methods Newly diagnosed AD suspects will undergo a lumbar puncture with CSF manometry, during neurological work-up. Ophthalmological evaluation consists of best corrected visual acuity, slit lamp biomicroscopy, gonioscopy, funduscopy and pachymetry. Diagnosis of glaucoma or ocular hypertension will be made on the basis of visual field examination, optic disc evaluation and IOP measurement. Correlation between CSF pressure, trans lamina cribrosa pressure gradient and the presence of glaucoma will be calculated. The prevalence of low tension glaucoma will be compared to the prevalence of chronic open angle glaucoma with elevated IOP. In the second part of the project a genetically modified strain of mice with AD will be examined and screened for the development of glaucoma. Ophthalmological examination will consist of IOP measurement, corneal pachymetry, optic disc evaluation and visual evoked potentials with flash. Histopathological analysis will be performed by the team of Prof De Deyn PP.

Results will follow

Conclusion will follow

■ 4134

The effect of microplasmin on wound healing after glaucoma filtration surgery

VAN BERGEN T
Leuven

Purpose The outcome of trabeculectomy can be diminished due to a decreased bleb function secondary to blood/ fibrin clot in the aqueous outflow pathway. The aim of this study is to investigate whether the administration of Microplasmin (ThromboGenics), a recombinant protein that dissolves clot and fibrin, could lead to a better maintenance of the constructed channel, and thus improve surgical outcome after trabeculectomy.

Methods The effect of Microplasmin will be investigated in vivo in a mouse model for conjunctival fibrosis and in a rabbit model for glaucoma surgery. Postoperative follow up of the animals will take place daily during the first week and two-daily until they are scarified. On specific time points animals will be sacrificed and both eyes will be enucleated. Seven-µm thin slides will be (immuno-)stained for CD45 to evaluate inflammation and for Sirius red and Trichrome to evaluate fibrosis.

Results Preliminary results showed that Microplasmin significantly improved glaucoma surgery outcome in the rabbit model of aggressive scarring compared to control.

Conclusion Our proposed research project will elucidate the potential role of Microplasmin in the improvement of filtration surgery outcome, and will highlight any anti-clotting, anti-inflammatory, and/or anti-fibrotic effects of this molecule. Microplasmin as an adjuvant therapy in glaucoma surgery might open new perspectives for more efficient surgery.

■ 4135

Study of the immune response in patients with uveitis and latent tuberculosis

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Purpose Mycobacterium tuberculosis infects up to 30 % of the population worldwide. In the majority of the cases a lifelong immune response, based on the production of IFN γ by CD4 $^{+}$ lymphocytes, restricts the infection into lung granulomas. A dysregulation of T regulatory cell function has also been implicated. It has been postulated that this constant immune response might contribute to certain forms of tuberculosis associated uveitis (hypersensitivity uveitis). The aim of this work is to analyse the lymphocyte production of IFN γ and the percentage of regulatory T cells in sight threatening uveitis patients with or without latent tuberculosis.

Methods Patients with sight threatening uveitis suspected to be related to tuberculosis or to autoimmune disease will be recruited at the CHU St-Pierre. Patients will be included if the work-up is compatible with the diagnosis of tuberculosis related uveitis or autoimmune uveitis used as a control. Signed informed consent will be obtained and blood samples will be taken.

Results IFN γ production in response to different mycobacterial peptides will be measured by QuantiFERON $^{\text{TM}}$ -TB Gold in-tube and by ELISA. IL-17 will be quantified by ELISA and the percentage of T regulatory cells analysed by flow cytometry (CD3 $^{+}$ CD4 $^{+}$ CD25 $^{\text{high}}$, CD127 $^{\text{low}}$, FOXP3 $^{+}$).

Conclusion The diagnosis of tuberculosis uveitis is a clinical challenge. The disease is probably mediated through infectious and immune mechanisms. By studying the CD4 $^{+}$ and regulatory T lymphocytes function in patients with uveitis and latent tuberculosis, we hope that we will better understand this pathology. In addition, this study will evaluate the usefulness of QuantiFERON $^{\text{TM}}$ -TB Gold in-tube in the evaluation of patient with uveitis.

■ 4141

The Sanger Institute mouse genetics programme: progress on the high throughput characterisation of knockout mice

GERDIN AK

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Purpose Anna-Karin GerdinThe Wellcome Trust Mouse Genetics Programme GroupWellcome Trust Sanger Institute, Genome Campus, Hinxton, Cambridgeshire, CB10 1SA, England, UK. Email: mousegenetics@sanger.ac.uk. The Sanger Institute Mouse Genetics Programme is committed to making a significant contribution to the functional annotation of the mammalian genome. We are utilising the growing knockout first conditional ready targeted ES cell resource currently being created by the EUCOMM and KOMP initiatives to generate, characterise and archive 250 lines of knockout mice per year, including 40 lines for the EUMODIC consortium. In addition to studying the role of each gene in normal development and function, the breadth and depth of our phenotyping platform ensures that phenotype information on a wide spectrum of disease conditions is obtained for each individual mouse line without the need for any prior assumptions about function. All phenotype data and biological resources generated by the programme are openly available to the scientific community.

Methods Eye morphology is routinely assessed using the Slit Lamp and Ophthalmoscope. Details are documented using a standardised checklist of observable characteristics. When abnormalities are identified, images are collected directly from the Slit Lamp or, for the retina, by using a topical endoscope (TEFI). Furthermore, we are in the process of assessing the Optokinetic Drum and its suitability in our high throughput phenotyping pipeline to measure visual perception and acuity. Here we report the progress on the Eye and Vision screens.

Results We present a summary of the phenotyping data available to date and examples of novel findings from a subset of interesting mutant lines.

■ 4143

Screening for mouse eye and vision defects at MRC Harwell

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Purpose Characterisation of novel genes involved in vision and eye development from the Harwell EUMODIC screen and a recessive ENU mutagenesis screen.

Methods Mice have been generated through the EuComm and EuModic programmes that have knockout mutations in defined genes. In addition, mice with random, undefined, mutations have been produced by chemical mutagenesis. Cohorts of these mice have been subjected to slit lamp examination and indirect ophthalmoscopy, and are tested for visual function using a virtual optokinetic drum with a computer generated image displayed around the test animal. Further phenotypic characterisation involves histology and use of tissue markers. In order to preserve the three-dimensional structure of the eye for examination of the vasculature, for example, we are exploring the use of Optical Projection Tomography which images the intact eye and allows reconstruction of eye structure.

Results We have identified a number of mouse lines defective in the visual system. These defects include microphthalmia, eyes open at birth, lens, cornea or iris defects, intraocular bleeding, and retinal degeneration. Furthermore, one line exhibits persistent fetal vasculature in the vitreous (PHPV) which normally regresses after birth through the action of resident macrophages. Determining the basis of this phenotype involves analysis of both the embryonic and adult eye, since mice with defective optic-fissure closure have also been described with this phenotype.

Conclusion Characterisation of these mutant lines will enable us to contribute to a more comprehensive understanding of the genetic regulation of eye development and tissue maintenance within the visual system. Many of these mutants are valuable genetic models of human eye diseases.

■ 4142

Recent findings and perspectives of the vision screen in the German Mouse Clinic

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Purpose The purpose of this study was the large-scale screening of different mouse models for eye disorders.

Methods The eyes of the mouse mutants were analyzed by slit lamp biomicroscopy, funduscopy, and laser interference biometry. Cataracts were quantified by Scheimpflug imaging.

Results In the past 15 months, 36 mouse mutant lines were screened in the German Mouse Clinic. Two of them, Ali030 and Fra2, were affected in eye development. Funduscopy and histology of Ali030 indicated an optic nerve head dysplasia due to an alternative splicing of the serine/threonine-protein kinase gene *Bmpr1b*. Fra2 is characterized by an H2-Kb promoter induced ectopic expression of the transcription factor gene *Fosl-2*. Laser interference biometric eye size measurements revealed a significantly reduced mean lens thickness in transgenic mice, indicating a putative role of *Fosl-2* in lens development. We further tested the clinically well established Scheimpflug imaging technique as a putative tool for cataract quantification in the vision screen. Measurements with the O377-crybB2 mouse mutant line successfully imaged even faint zonular opacifications. Furthermore, a core density of 40% and a peripheral opacification between 10% and 14% was calculable. Our results demonstrated that Scheimpflug imaging is an improved quantitative alternative to subjective slit lamp investigations.

Conclusion Two novel mouse models for optic nerve head dysplasia and microphakia were detected in the vision screen. Scheimpflug imaging was demonstrated to be suitable for cataract quantification in the mouse lens.

■ 4144

Visual phenotyping at the Institut Clinique de la Souris

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Visual diseases come in many flavors, with a large variety of affected tissues (eye anterior segment, retina, optic nerve, cortex ...), ages of onset, rates of progression and causal factors. In Western countries, if the majority of these diseases are now curable, millions of people are still affected by blindness or low vision, as many retinal diseases (age-related macular degeneration, retinitis pigmentosa, diabetic retinopathy, glaucoma...) still lack efficient treatments. In a facility devoted to mouse phenotyping, it is thus of major importance to propose an efficient visual phenotyping platform, to pick up visual defects in screened mutants, to assess the beneficial effects of potential treatments or the eventual adverse effects of drugs targeting the CNS – vision screen being mandatory in CNS drug development. While the tests performed in human clinics are transposable to rodents, the small eye size of the mouse hinders a fine assessment of the retinal fundus, especially in the periphery. To maximize our chances to detect even early phenotypes, we are moving toward a recently developed fundus imaging system (Paques et al., IOVS 2007), which allows to acquire high quality digital images of the fundus, up to the ciliary margin, and fluorescein angiography, allowing for a later diagnostic confirmation by ophthalmologists. Together with slit lamp examination and electroretinography, this constitutes a solid first line of visual phenotyping.

■ 4151

Precise monitoring of intraocular inflammation by LFP: the gold standard

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Purpose Laser flare photometry is an objective quantitative method that enables accurate measurement of anterior chamber flare with a very high reproducibility.

Methods Review of literature

Results Clinical studies of uveitis patients have shown that flare measurements by laser flare photometry allowed precise monitoring of well defined uveitic entities and prediction of disease relapse. It is useful both in acute and chronic forms of intraocular inflammation. Sensitivity of laser flare photometry has been shown in posterior uveitic entities as well. Relationships of laser flare photometry values with complications of uveitis and visual loss have been reported.

Conclusion Flare measurement by laser flare photometry should be included in the routine follow-up of patients with uveitis.

■ 4153

Gain in accuracy for diagnosis and follow-up of uveitis through indocyanine green angiography

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Purpose The proportion of diseases involving principally the choroid is as frequent if not more frequent than those involving the superficial structures of the fundus and are therefore not accessible or poorly accessible to fluorescein angiography (FA) nor OCT.

Methods Illustrative cases where the preponderant lesions are in the choroid are presented showing that the detection and follow-up of choroiditis is only meaningful using indocyanine green angiography (ICGA).

Results Diseases that do not involve the choroid are the minority. Except for intermediate uveitis, mainly of the pars planitis type, and Behçet's uveitis, choroidal involvement cannot be excluded. Therefore in most other uveitis cases with suspected posterior involvement, if angiographic investigation is felt necessary, the initial angiography should always be a dualFA/ICGA angiography even if the predominant fundus sign is retinal vasculitis. Only if the initial angiography does not show choroidal involvement can the follow-up be performed by FA alone.

Conclusion In order to perform adequate diagnosis, adequate assessment of lesions and adequate follow-up in uveitis cases with choroidal involvement ICGA is mandatory.

■ 4152

Progress in monitoring inflammation in JIA

BODAGHI B

Paris

Purpose To analyze the most appropriate strategy to monitor ocular inflammation in children with juvenile idiopathic arthritis - associated uveitis.

Methods Slit lamp biomicroscopy is widely used to evaluate the importance of anterior segment flare and cells in children with anterior uveitis. However, different studies have clearly shown that other tools such as laser flare photometry and OCT may improve the monitoring during the follow-up. Therefore, both tests are performed in all children referred to our Department for the management of JIA-associated uveitis.

Results Laser flare photometry showed for the first time that active ocular inflammation may be associated with a significant level of flare even in the absence of detectable cells. This is a major finding to start a therapeutic approach or change it for a more aggressive strategy and monitor the decrease of flare. Moreover, the level of flare decrease under therapy may predict further serious complications such as secondary glaucoma or cataract. OCT features are important to identify macular alterations in nearly 85% of children with anterior uveitis associated with JIA.

Conclusion Both laser flare photometry and OCT are non invasive and quantitative methods that may significantly improve the visual outcome of JIA-associated uveitis.

■ 4154

Global angiographic scoring system for inflammatory diseases

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Purpose Fundus fluorescein and indocyanine green angiography are essential imaging techniques in the appraisal of posterior segment inflammation. A combined fluorescein and indocyanine green angiographic scoring system has been developed in order to provide semi-quantitative data for follow-up of disease progression, monitoring response to treatment, and comparison between clinical studies. We tested interobserver variations in the semi-quantitative scoring of dual fluorescein/indocyanine green angiograms.

Methods Four observers scored 32 dual fluorescein and indocyanine green angiograms. Spearman rank correlation was used to analyze correlation between scores assigned to each angiographic sign. We used the Kappa statistics to test agreement between pairs of observers in comparative total fluorescein and indocyanine green angiographic scores.

Results We found a significant correlation between pairs of observers in scores assigned to each fluorescein angiographic sign and the total score of fluorescein angiograms. A significant correlation was found only between 2 separate pairs of observers in scores assigned to early stromal vessel hyperfluorescence on indocyanine green angiography. However, a significant correlation was found in other indocyanine green angiographic signs and the total score of indocyanine green angiograms. There was a good agreement between observers in comparative fluorescein – indocyanine green angiographic total scores.

Conclusion Further experience with the scoring system, especially with the indocyanine green angiographic scoring, may improve its reproducibility.

■ 4155

Progress in monitoring inflammatory CMO

DESMET M

ABSTRACT NOT PROVIDED

■ 4156

Progress in the appraisal and management of inflammatory CNVs

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Purpose To review the current Literature and to describe the experience of a tertiary referral centre on the progress in the appraisal and the management of inflammatory choroidal neovascularization (CNV).

Methods The current literature is reviewed and the experience of a tertiary referral centre is reported.

Results CNV is a potentially severe sequela of posterior uveitis. The role of chronic inflammation has been described in experimental uveitis. For such reasons, even when biomicroscopy and fluorangiography (FA) cannot detect abnormalities, Indocyanine Green Angiography (ICGA) can show choroidal anomalies. ICGA greatly improved the appraisal of the choroidal involvement, by providing reliable data for the diagnosis and for the management of inflammatory CNV. The new spectral domain optical coherence tomography (OCT) equipments can provide further informations that can be useful for a correct clinical assessment. The out-come of subfoveal CNV is poor if untreated: several procedures have been considered, even though there is lack of guidelines. Steroids, both local and systemic, are the first line therapy for non-infectious choroidal inflammation, although their long-term use can lead to unpleasant sequela, such as glaucoma and cataract. Immunesuppressive agents, lasers photocoagulation, photodynamic treatment, surgical removal and anti-Vascular Endothelial Growth Fact (VEGF) are other options.

Conclusion CNV secondary to uveitis is a severe sequela leading to significant visual impairment. ICGA is mandatory in order to obtain relevant informations about the choroidal status. Several therapeutic options have been considered, but no guidelines are available at the moment.

■ 4161

Metastatic disease in small uveal melanomas: retrospective review of 368 patients

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Purpose To determine the metastatic rate and survival curves of small uveal melanomas and find the smallest uveal melanoma associated with metastatic disease.

Methods We studied uveal melanomas patients treated with radiotherapy in Curie Institute between 1992 and 2004. We selected the tumors with a diameter inferior or equal to 12 mm and a thickness inferior or equal to 3 mm. All the datas concerning initial tumor findings, radiotherapy treatment and follow up were routinely entered in the data base. Retrospective review and statistical analysis were performed.

Results Among 2258 patients treated during this period, 368 had small tumors. Median tumor diameter was 9 mm and median tumor thickness was 2,5 mm. Retinal detachment was present in 32 patients. 282 patients were treated by proton beam therapy, 77 by iodine plaque and 9 by transpupillary thermotherapy. Median follow up is 109 months. 71 patients died and 20 patients developed metastatic disease. Local recurrence was observed in two cases. Overall survival at 5 years was 92% and at 10 years 78% survival without metastasis at 5 years was 96% and at 10 years 93%. According to our data the smallest tumor associated with metastatic death was 5mm in diameter and 1,5 mm in thickness and 14 of the tumors had a diameter of less than 10 mm developed. Half of the metastatic patients developed metastasis 5 years or more after treatment.

Conclusion Very small uveal melanoma can be responsible for metastatic death.

■ 4163

Uveal melanoma: management and outcome of patients with extraocular spread

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Purpose Extraocular spread is thought to be a negative prognostic factor on survival of the patient with uveal melanoma. Depending on the size of the tumor and the type of extraocular extension conservative treatments can be employed.

Methods 2256 patients were treated between 2000 and 2007 at the Institut Curie, Paris, France for an uveal melanoma. 67 patients (3.0%) presented an extraocular extension. A retrospective study was performed to evaluate the patients outcome with regard to tumour recurrence and their survival.

Results Results: Eye-conserving treatment was employed in 38 (52.8%) patients. An enucleation was performed in 29 (47.2%) patients. The median follow-up was 38 (range 7 - 79) months with an overall survival rate at 5-years of 40.4% in enucleated patients and 79.3% in the eye-conserving treatment group (protons n=19, iodine-125 plaque n = 19) (p = 0.01; Kaplan-Meier analysis). No tumor recurrence was observed in any group. Degree of extraocular spread as well as the clinical characteristics tumor location, retinal detachment, ciliary body involvement (p < 0.01; Chi-square test) and tumor thickness (p = 0.04; Chi-square test) influenced the choice of treatment. Age, tumor diameter, involving optic nerve, vitreous hemorrhage, achromic lesion was without any influence.

Conclusion Conclusion: No tumor recurrence and no lower survival rate were observed in patients receiving an eye-conserving treatment. They may represent thus a therapeutic option in selected patients with extraocular spread.

■ 4162

Clinical presentation, pathological features and natural course of metastatic uveal melanoma (MUM) as an orphan and commonly fatal disease

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Purpose Uveal melanoma (UM) is a rare disease characterized by an unpredictable course and variable outcome ranging from cure by local treatment to the occurrence of untreatable metastasis. The current project is focused on the characteristics of the metastatic phenotype of the disease

Methods We performed data collection from 76 pts with MUM treated in Leuven between 1957-2008. Statistical analysis involved nonparametric technics, Kaplan Meyer and log rank test

Results The median age at diagnosis of UM was 58 yrs (range 30-94). Common initial treatments were surgery (71%), brachytherapy (20%) and external beam RT (7%). Synchronous metastasis was found in only 9% of cases, all others had metachronous disease after a median interval of 40 mo (range, 7-420). Metastasis in >1 organ, was seen in 47% of cases. The most frequent metastatic site was the liver (96%), followed by lung, subcutaneous, bone and brain lesions. The median OS from diagnosis of UM was 46 months (range, 2-182), and only 4,5 months in pts with MUM (range, 1-128). 65% of MUM pts qualified for further treatment. The most common drugs given were DTIC, cisplatin, tamoxifen or phase I agents. Patient benefit (PR+SD) was seen in 16/45 pts (36%), including 2 PR

Conclusion In this orphan disease with female predominance metastasis occurs late, is mainly found to the liver, and is associated with high morbidity, as >1/3 of pts do not qualify for further therapy. Advances in MUM can only be achieved by networking of sites interested in this tumour type with systematic collection of data and tissue to improve our understanding of the molecular biology of the disease

■ 4164

Cytogenetic profile of locally invasive posterior uveal melanoma

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Purpose To analyze cytogenetic profile of locally invasive posterior uveal melanoma (UM).

Methods Twenty consecutive cases of large posterior UM with histopathologically confirmed extrascleral extension were included in this non-comparative cases series. Fine needle aspiration biopsy (FNAB) of the intraocular tumor portion was performed using 25-gauge trans-scleral approach, immediately after enucleation of the globe. FNAB of the extrascleral tumor portion was also performed when it was > 1mm in thickness. Sampled material underwent fluorescence in situ hybridization (FISH). Follow-up was longer than 12 months.

Results Six tumors (30%) had both intraocular and extraocular tumor samplings, whereas 14 tumors (70%) showed extrascleral extension less than 1 mm in thickness. Monosomy 3 was found in 5 tumors (25%), whereas disomy 3 in 15 tumors (75%). Cytogenetic profile of the intraocular tumor portion appears to be maintained in the extrascleral extension in all cases (100%). Five patients (25%) developed metastatic disease during follow-up (all had monosomy 3 tumors).

Conclusion Extrascleral extension appears more frequent in disomy 3 tumors. Cytogenetic profile of locally invasive posterior UM is maintained in the extrascleral tumor portion and must be considered the most important prognostic factor in locally invasive tumors.

■ 4165

Does delayed treatment shorten the life of patients with fatal choroidal melanoma?

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Purpose Metastatic death from uveal melanoma occurs in about 50% of patients many of whom experience a delay in treatment, either intentionally or accidentally. The aim of this study was to determine whether treatment delay shortens survival in patients with choroidal melanoma whose disease apparently proved fatal.

Methods Patients with choroidal melanoma were included in the study if resident in mainland Britain and if deceased. Survival was analysed according to basal tumour diameter by Kaplan-Meier and Log rank analysis.

Results A total of 696 patients with choroidal melanoma died. The patients had a median age of 65 years and a median basal tumour diameter of 15.0 mm. The basal tumour diameter was <10mm in 41 patients; 10-11mm in 88; 12-13mm in 108; 14-15mm in 165; 16-17mm in 123; and >17mm in 171 patients. Log-rank analysis showed no correlation between survival and basal tumour diameter in these patients (Log rank analysis, $p = 0.5537$). There was perhaps a trend towards longer survival in patients with a basal tumour diameter less than 10mm.

Conclusion In patients with fatal uveal melanoma, there is no significant correlation between basal tumour diameter and survival time. Delay in treatment does not seem to worsen prognosis for survival significantly, except perhaps in patients with small tumours. This finding adds further support to the concept that the main objective of ocular treatment is to conserve the eye with as much useful vision as possible. Since ocular treatment can itself cause significant visual loss, the benefit of treating asymptomatic uveal melanomas is uncertain. There is scope for randomized, prospective studies of treatment versus non-treatment of patients with asymptomatic choroidal melanoma.

■ 4167

Primary photodynamic therapy of choroidal melanoma

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Purpose To review our initial experience with photodynamic therapy of choroidal melanoma at the Ocular Oncology Service in Liverpool.

Methods Patients were included in the study if they underwent primary photodynamic therapy for choroidal melanoma. The treatment was administered using the same protocol as for choroidal neovascularization.

Results The patients (12 male and 5 female) had a mean age of 62.2 years. The melanomas were located in the right eye in 11 patients and the left eye in 6 patients. The tumour margin extended anteriorly to pre-equatorial choroid in one patient and posteriorly to include optic disc in 7 patients. The melanomas had a mean diameter of 7.6 mm and a mean thickness of 2.0mm. The initial visual acuity was 6/12 or better in 13 patients and 6/18-6/60 in 4 patients. Biopsy showed the tumour to be of spindle cell type in two patients and to contain epithelioid cells in three patients. One patient was found to have monosomy 3 so that the tumour was treated with proton beam radiotherapy. The follow-up ranged to 622 days with a median of 101 days. Four patients subsequently underwent proton beam radiotherapy and one patient was treated by endoresection. The last known visual acuity was 6/12 or better in 11 patients, 6/18 to 6/60 in 4 patients and 3/60 to Counting Fingers in 2 patients.

Conclusion Photodynamic therapy may be worth attempting in patients with a small choroidal melanoma when other methods are likely to cause visual loss. Many patients subsequently require more aggressive treatment to achieve local tumour control.

■ 4166

An audit of eccentrically-positioned ruthenium plaque radiotherapy of choroidal melanoma in Liverpool

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Purpose Brachytherapy is usually administered with the plaque overlapping the entire tumour margin by at least 1-2mm. With posterior tumours, our practice is to position the plaque with its posterior edge aligned with the posterior tumour margin. We audited ocular outcomes after eccentrically-placed ruthenium plaque radiotherapy of choroidal melanoma.

Methods Patients were included if receiving primary ruthenium brachytherapy for choroidal melanoma during the three years up to the 31st July 2007. A perforated template was used to facilitate plaque positioning. For posterior tumors, the template was positioned so that trans-illumination produced a glow at the posterior tumour margin ('sunset sign'). Minimum doses of 300-350 Gy and 80-90 Gy were prescribed to the sclera and apex, respectively.

Results The cohort comprised 162 patients (93 female and 69 male). The time to the last known visual acuity had a median of 23 months. The initial visual acuity was 20/40 or better in 94.6%, 20/60 to 20/200 in 13.0% and worse than 20/200 in 1.9% of patients. The tumours had a mean basal diameter of 11.7mm. Ten tumours exceeded 5.4 mm in height. Tumour extension to within 5mm of optic disc, fovea or both occurred in 18 (11.1%), 28 (17.3%) and 27 (16.7%) cases respectively. Risk factors for visual loss were proximity to optic disc or fovea, initial visual acuity worse than 20/40 and tumour height exceeding 5.4 mm. In 66 patients with none of these risk factors, 92% retained 20/40 or better and 5 had vision of 20/60 – 20/200. In 72 with one risk factor, 74.3% retained 20/16 – 20/40 and 95.7% had vision of 20/200 or better. In 12 patients with 2 risk factors, these percentages were 25.0% and 91.7%. Only 3 patients had 3 risk factors and one retained vision of 20/200 or better. Tumours distanced < 5 mm to fovea were divided in 3 groups, and visual acuity analysed. Three patients had local tumour recurrence and were treated respectively by proton beam radiotherapy, plaque radiotherapy and enucleation (the only eye lost in this series).

Conclusion Eccentric plaque radiotherapy of choroidal melanoma achieves good rates of local tumour control, ocular retention and preservation of vision.

■ 4168

Open-sky biopsy of ciliary body or choroidal tumors of undetermined origin: utility and safety

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Purpose In the presence of a ciliary body or choroidal tumor of undetermined origin, an open-sky biopsy is performed in selected cases to establish a diagnosis and specify the therapeutic approach. We explored the frequency and reliability of this diagnostic procedure.

Methods Retrospective, consecutive, histopathologic case series of 30 biopsies since 1989 of ciliary body or choroidal tumors of undetermined origin.

Results Eighteen tumors originated from the ciliary body, 12 from the choroid. Diagnosis was respectively adenoma/adenocarcinoma (4/0), mesectodermal leiomyoma (4/1), metastatic tumor (2/0), melanocytic tumor (4/5), lymphoma (1/3); hemangioma (1/0) or posterior nodular scleritis (0/3). In 2 cases, there was insufficient material to make a diagnosis. There were no cases where the biopsy complicated local or systemic tumor control.

Conclusion Open-sky biopsy is only performed in cases whose clinical appearance doesn't permit to confidently establish a diagnosis; its goal is to guide the therapeutic approach.

■ 4211

Semi-automated assessment of microaneurysm formation rate from color fundus photographs in patients with mild NPDR

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Purpose To assess the reliability of microaneurysm (MA) formation rate, computed from color fundus photographs, in type-2 diabetic patients with mild NPDR.

Methods In a 2-years observational and prospective study of 400 type-2 diabetic patients with mild NPDR, MA formation rate (MAFR) was computed using a new method (MA-Tracker). This method allows graders to earmark MAs location on color fundus photographs. By using image co-registration, it became possible to track previously identified MAs and to identify newly earmarked ones. The reliability of the procedure, assessed using 3 independent graders and a total of 235 color fundus photographs from a previous dataset, demonstrated a very good agreement between graders for the number of MAs (ICC=0.964) and for the MAFR (ICC=0.981).

Results Sixty-three of the 400 patients/eyes that completed the baseline and the 6-month visits had MAFR computed using the MA-Tracker. While the number of MAs earmarked at baseline (mean±SD: 2.0±2.5 MAs) and at 6-months (2.7±3.7 MAs) showed no statistically significant increase (P=0.105), the MAFR, on the other hand, showed a high MA turnover (mean±SD: 2.7±3.7 new MAs for this 6-months period of follow-up).

Conclusion Microaneurysm formation rate obtained from color fundus photographs using the MA-Tracker show a very good reliability between graders making it a new tool with potential to be used as a biomarker of DR progression.

■ 4213

Retinal hemodynamic changes in diabetic macular edema

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Purpose To report the 1 year prospective hemodynamic results in a cohort of patients with varying levels of risk for the development of diabetic macular edema (DME) and to correlate these parameters to systemic changes in blood pressure and diabetes control.

Methods The sample comprised 4 groups. Group 1: 37 non-diabetic controls; Group 2: 42 patients with no diabetic retinopathy (DR); Group 3: 38 patients with DR but no DME; Group 4: 27 patients with DME. Retinal arteriolar diameter, velocity, maximum-to-minimum (max:min) velocity ratio and flow were measured using the Canon Laser Blood Flowmeter. Blood pressure, blood and urine tests were taken and correlated to changes in retinal hemodynamics. A sub-group of patients with progressive DR was analyzed separately.

Results There was a trend for elevation of the max:min velocity ratio (p=0.060) with increasing risk of DME at baseline which was significant on follow-up (p=0.020). Diameter decreased for all groups (p<0.056) except for patients with DME. Changes in retinal blood velocity, max:min velocity ratio and flow were correlated to blood pressure mostly in patients with DR and DME. Blood albumin level was negatively correlated to retinal blood velocity and flow in the DME group. Patients with progressive DME had elevated blood velocity and flow.

Conclusion Patients with DME have a higher max:min velocity ratio. Changes in retinal hemodynamics were correlated to changes in blood pressure and were negatively correlated to changes in blood albumin. The results indicate a loss of vascular compliance with diabetes, particularly in patients with DME, and elevated retinal perfusion in patients with progressive DME.

■ 4212

Validation of a predictive model for diabetic retinopathy progression in type-2 diabetic patients with mild nonproliferative diabetic retinopathy.

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Purpose To validate a predictive model for diabetic retinopathy progression in type-2 diabetic patients with mild nonproliferative diabetic retinopathy (NPDR) using non-invasive examinations, previously reported by Lobo et al. [Arch Ophthalmol. 2004; 122(2):211-7].

Methods Four-hundred type-2 diabetic patients with mild NPDR were included in this 2-years observational and prospective study. Sixty-three patients performed the baseline and the 6-month visits undergoing: color fundus photography, retinal thickness (RT) measurements and blood tests. Microaneurysm formation rate (MAFR) was computed from color fundus photographs using a new method that assists graders on earmarking MAs (MA-Tracker). Increased RT maps (Stratus OCT – Carl Zeiss Meditec, Dublin, CA, USA) were computed using proprietary software. Patients were classified into one of the 3 different phenotypes of DR progression characterized by: Phenotype 1 – eyes with low MAFR and normal RT; Phenotype 2 – eyes with increased RT and; Phenotype 3 – eyes with high MAFR.

Results Twenty-six patients (41.6%) were classified as being of Phenotype 1; 17 (27.0%) as Phenotype 2 and; 20 (31.7%) as Phenotype 3. This distribution of patients in each phenotype is similar to the one previously found when establishing the predictive model.

Conclusion Preliminary data confirms the existence of 3 different phenotypes of DR progression.

■ 4214 / 455

Long-term result of intravitreal steroid for macular oedema: 2 to 5 year follow-up of 92 eyes

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Purpose A topical area is how best to manage diabetic macular oedema (intravitreal steroid, laser, anti-VEGF). However little or no long-term data exists for steroids. We hence studied the 2 to 5-year results of intravitreal triamcinolone (IVTA) in diabetic macular oedema.

Methods A retrospective cohort of 92 eyes was recruited, inclusion criteria subjects with diabetic macular oedema injected with 4mg / ml IVTA with regular follow-up of 2 to 5 years. Exclusion criteria were subjects with non-diabetic oedema (uveitis, vascular, post-operative) and baseline foveal ischaemia at time of first injection. The following were recorded: visual acuity, central retinal thickness (OCT) prior, 1 month after (+/- 1 week) and 3 months post-IVTA (+/- 2 weeks), complications, and angiograms pre- and post-treatments. 88% had Type 2 diabetes. Mean duration of diagnosis of diabetes was 14 years, mean age 64 (29-84).

Results At 2 to 5 years IVTA continued to be effective both in improving visual acuity and reducing macular thickness in 76% of subjects, including after multiple injections. In 24% of subjects foveal ischaemia limited outcome, usually 36 to 54 months post initial treatment. In 8% of subjects a repeat injection was associated with cessation of leakage for 2 to 3 years, or a persistent marked diminution in leakage of in excess of 100 microns, thereafter vision dropping to 6/12 to 6/18 despite further injections. Intra-ocular pressure rises were controlled topically, there was one case of sterile endophthalmitis.

Conclusion IVTA continues to offer significant visual benefit and reduction in macular thickness 2 to 5 years after initiation of therapy, including after multiple injections. Therapeutic potential is limited by ischaemia in 24% of subjects.

■ 4215 / 456

The relationship between retinal hemodynamics and systemic markers of endothelial function and inflammation in type 2 diabetes

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Purpose The aim of the study was to investigate the associations, if any, between retinal hemodynamics and systemic markers of endothelial function and inflammation in patients with type 2 diabetes and non-proliferative diabetic retinopathy (NPDR).

Methods Retinal blood flow measurements and blood serum samples were obtained from 9 healthy controls (Group 1, mean age 53.8 yrs, SD 11.7), 23 patients with mild-to-moderate NPDR (Group 2, mean age 61.4 yrs, SD 8.5; duration 9.9 yrs, SD 8.8) and 14 patients with moderate-to-severe NPDR (Group 3, mean age 60.3 yrs, SD 9.9; duration 17.0 yrs, SD 9.4). Retinal blood flow was measured in the supero-temporal arteriole using the Canon Laser Blood Flowmeter. Blood samples were collected to derive levels of intercellular adhesion molecule (ICAM-1), vascular adhesion molecule (VCAM-1), E-selectin and von Willebrand factor (vWF).

Results Diameter, velocity, maximum-to-minimum velocity ratio and flow were the same across the groups and did not change over a 6 month follow-up. A1c and ICAM-1 were significantly elevated across the groups ($p=0.001$ and $p=0.030$, resp., ANOVA). VCAM-1 ($r=0.325$, $p=0.046$) and vWF ($r=0.334$, $p=0.050$) showed relatively weak associations with baseline max:min velocity ratio. vWF showed moderate associations with change in velocity ($r=0.571$, $p=0.007$) and change blood flow ($r=0.455$, $p=0.038$).

Conclusion The relationship between endothelial dysfunction and inflammation markers and retinal hemodynamics in type 2 diabetes is complex since not all markers show an association. This work is on-going.

■ 4217 / 458

Effects of conventional argon panretinal laser photocoagulation on retinal nerve fibre layer and driving visual fields in diabetic retinopathy

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Purpose To determine the effects of argon green panretinal laser photocoagulation (AG-PRP) on retinal nerve fibre layer thickness (RNFLT), threshold visual fields (VF), and Estermann full binocular VF over time.

Methods Prospective, pilot clinical study of patients with newly-diagnosed proliferative diabetic retinopathy (PDR). Time-Domain optical coherence tomography (TD-OCT) of optic nerve head and 24-2 SITA-Fast Humphrey/Estermann VF (HVF, EVF) recorded at baseline, 10 weeks and 6 months post-laser. Quantitative field analysis of central 10°, 24°, and binocular VF.

Results 10 eyes underwent uncomplicated multiple-session 100ms AG-PRP using 2000 burns, 400µm spot, and mean power 136 mW (SD 39.3). TD-OCT detected and quantified an increase in mean RNFLT at 10 weeks (+8 µm; $p<0.05$) and progressive thinning at 6 months (-4 µm; $p<0.05$) compared to baseline. Mean threshold sensitivities, 10° and 24°, improved at both time-points in the majority (9/10 and 8/10) of patients. Masked grading of EVFs showed no significant change with treatment.

Conclusion This pilot study demonstrates that conventional AG-PRP may increase the RNFL in the short-term, presumably related to laser-induced axonal injury, with progressive thinning of RNFL in the longer-term. The sensitivity of both 10° and 24° VFs improved significantly following AG-PRP, and this central functional improvement may be due to a reduction in oedema following AG-PRP. Binocular standard driving VFs performed within 6 months of AG-PRP may actually reflect pre-existing VF abnormalities due to severe retinal ischaemia or non-viable retina at presentation, rather than direct functional loss from laser.

■ 4216 / 457

Assessment of retinal arteriolar hemodynamics in patients pre- and post-cataract extraction

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Purpose We have previously demonstrated that artificial light scatter results in the erroneous elevation of retinal vessel diameter and blood flow using densitometry based techniques. The aim of this study was to determine the impact of cataract on the quantitative, non-invasive assessment of retinal arteriolar blood flow.

Methods Of the original 30 recruited patients who were scheduled for extra-capsular cataract extraction using phacoemulsification and intraocular lens implantation, ten patients between the ages of 61 and 84 (mean 73 ± 8) successfully completed the study protocol. Two visits were required to complete the study, one prior to the surgery and one at least six weeks after the surgery. Cataract status was documented using the Lens Opacity Classification System (LOCS, III) on the first visit. Retinal arteriolar hemodynamics were measured using the high intensity laser setting of the Canon Laser Blood Flowmeter (CLBF) on each visit.

Results Group mean retinal arteriolar diameter and blood flow were significantly lower following extracapsular cataract extraction (Wilcoxon signed-rank test, $p=0.022$ and $p=0.028$ respectively); however, centreline blood velocity was unchanged (Wilcoxon signed-rank test, $p=0.074$). The primary reason for failure to complete the study protocol was due to poor retinal image quality impairing CLBF measurement.

Conclusion Densitometry assessment of vessel diameter is extraneously impacted by the presence of cataract. Care needs to be exercised in the interpretation of studies of retinal vessel diameter and blood flow that utilize densitometry techniques.

■ 4221

**How should we quantify the performance of KPro's?
The Visual Acuity by Time –Index (VATI)**

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Purpose To report a method of standardized data collection and reporting and statistical assessment that can be used for all KPro's available on the market.

The database (will be presented) should be

- Usable for different types of KPro's
- Easily adaptable to changes in technique
- Allow for complete entry of relevant data

Methods Visual Acuity data should be reported in different international surgical centres in a standardized manner: Best spectacle corrected VA, unless BCVA only possible with CL (> useful time of wear). A complete entry of all relevant data is possible in this database. The statistical analysis should be agreed upon by all centres. For Survival Time = Retention of KPro > the Kaplan-Meier method For Visual Acuity over the Course of Time > the Monte-Carlo method

Results A database will be demonstrated that can be used free of charge by all KPro centres interested. The VISUAL ACUITY BY TIME- INDEX (VAT- Index) will also be presented, whose theoretical basis published in: Journal of Theoretical Medicine, 2002 / 4, 183-190, W. Hitzl and G. Grabner „Application of the Monte Carlo Method for the Assessment of Long-term Success in Keratoprosthesis Surgery“. Example of its use will be given, based in data, courtesy Barraquer Eye Clinic, Barcelona.

Conclusion With the Kaplan-Meier method:

- + analysis is done quickly, uses all data available, hypotheses tests are available for comparisons and mean and median survival time can be computed
- no information about relation between time and best corrected visual acuity and the definition of terminal event is arbitrary to a certain extent.

Monte-Carlo method (VAT-index):

- + Method is based on a so-called non-parametric longitudinal model
- + Reliabel estimation of relation between time and best corrected visual acuity at any given time point (patient as well as surgeon is basically interested in this relation).
- + statistically valid analysis and better comparison of different KPro techniques
- + easy comparison of defined postoperative periods
- + comparison of different initial clinical findings and diseases possible
- + long-time follow-up of BCVA
- shorter follow-up time as compared with Kaplan-Meier method (e.g. with strict „80% data complete“ criteria)

■ 4223

Boston keratoprosthesis in pediatric patients

AQUAVELLA J

ABSTRACT NOT PROVIDED

■ 4222

The Boston keratoprosthesis in autoimmune disease

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Purpose Patients with corneal blindness due to mucous membrane pemphigoid and Stevens Johnson syndrome who undergo corneal transplantation carry a poor prognosis for visual recovery. The Boston keratoprosthesis has been demonstrated to provide excellent retention rates and postoperative visual acuity in patients with corneal graft failure, however, poor visual outcomes still occur in patients with underlying autoimmune disease.

Methods We reviewed the current literature to determine the results of keratoprosthesis in patients with blinding autoimmune diseases.

Results Much of the published literature on keratoprosthesis fails to clearly differentiate outcomes on the basis of the underlying disorder. Based on available evidence, inflammation, retinal detachment, and glaucoma appear to be the most significant complications after keratoprosthesis in autoimmune patients, and a diagnosis of mucous membrane pemphigoid or Stevens Johnson Syndrome appears to be associated with a significantly higher complication rate than other preoperative conditions.

Conclusion Patients with autoimmune diseases carry the worst prognosis for success with keratoprosthesis. Improvement in clinical outcomes might be achieved with changes in keratoprosthesis design and material, perioperative therapy, and/or surgical technique. Possible approaches to complications after Boston keratoprosthesis in patients with underlying autoimmune diseases will be discussed.

■ 4224

Long-term functional and anatomical results of OOKP and tibial OKP: Barcelona experience

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Purpose To report the long-term functional and anatomical results of OOKP and Tibial OKP performed at the Barraquer Eye Center from 1974-2006. Different factors like surgical technique, clinical diagnosis, age and post-operative complications are also analyzed and reported.

Methods A retrospective study on 330 eyes of 227 patients who underwent OOKP or tibia OKP was performed. Kaplan Meier survival curves and multivariate analysis using Cox regression model of the different variables mentioned are presented.

Results OOKP and Tibia OKP have comparable functional and anatomical results in the long-term. Best long-term functional and anatomical results are for patients with chemical burns, cicatricial trachoma and Stevens-Johnsons/Lyell syndrome. Thermal burns have a higher anatomical retention than other diagnostic categories. Younger patients have better visual prognosis and anatomical retention than older patients. Patients must be warned of the possibility of sight-threatening complications like extrusion of the prosthesis, retinal detachment and glaucoma.

Conclusion Our more than 35 years experience with biological keratoprosthesis using the OOKP and the tibia OKP shows that the two techniques are comparable. Certain variables like clinical diagnosis, age and post-operative complications affect the anatomical and functional results in the long-term.

■ 4225

Tibial KPro

TEMPRANO J
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The clinical results, from the anatomical point of view, are very good, although in the cases of mucosynechial syndromes, which also affect the buccal mucosa, pemphigoids or necrolysing epidermosis (Lyell), the mucosa may retract or necrolyse. In these cases we use the transtarsal method. In elderly patients with osteoporosis the bone may be resorbed and it would be necessary to repeat the procedure using bone from a more favorable area of the tibia, which should be determined with local densitometry. As far as the functional results are concerned, these depend on the conditions of the retina and the optic nerve. If both are in good conditions and the ocular pressure is normal, 20/20 vision may be achieved, although the visual field will never be more than 30° maximum. Indications: We perform this type of keratoprosthesis in patients without suitable teeth, affected with: Physical caustications (metal burns, bomb explosions, fire burns, etc.) Chemical caustications, no matter whether the origin be alkali (worse prognosis because of the glaucoma which usually develops in these cases) or acids (lime, sulphuric acid, sodium hydroxide, etc.) Important trauma where the eye had to be covered with skin because of extensive lid destruction. Advanced trachoma with total leucoma and extensive vascularization. Pemphigus and all mucosynechial syndromes which lead to total xerosis. Lyell's syndrome, epidermizing necrolysis. Cases of repeated failure of corneal transplantations. Anterior segment alterations due to complicated aphakia, bullous keratopathy affecting the entire cornea after previous failure of keratoplasty. Generally, total leucoma with important alterations of the limbus which would not permit survival of a corneal homotransplant.

■ 4227

AlphaCor, Pintucci, Supradescemetic

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Purpose To report the long term results of a supradescemetic corneal implant to restore vision

Methods A Keralia "soft" keratoprosthesis was implanted in one eye of a seventy years old woman bilaterally blind and affected by a bilateral limbal stem cells deficiency.

Results After four years the implant is still insite, well tolerated. The initial visual recovery has been compromised, during the last year, by a progressive opacification of the superficial layer of the device.

Conclusion The Keralia supradescemetic implant seems well tolerated in the long term by the human eye. Problems related to the stability of the material should be solved in future.

■ 4226

MOOKP clinical results and Indications

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Purpose To demonstrate which are the reasons that permit by the modified osteo-odonto-keratoprosthesis (Mookp) technique to obtain in 275 blind eyes, with a basic pathology where 42% of eyes were dry, and 32% had severe physical/chemical burns, excellent anatomical and functional results.

Methods Tables are shown which put into evidence the best visual acuity obtained in the 275 eyes affected by corneal blindness, operated by the Mookp technique from 1973 to 2008, with a 36 years causticity, with a very long follow-up.

Results 275 blind eyes. Best Visual Acuity recovered, between 10/10 and 5/10 in 80.25% of operated eyes, excellent for any working activity, (best visual acuity recovered), between 10/10 and 2/10 in 91.20%, useful for any activity. Final V.A., between 10/10 and 5/10 in 67.68%, and between 10/10 and 2/10 in 78.11%.

Conclusion The reasons for these excellent results are due to the biological properties of this Kpro invented by Strampelli and to the modifications made by Falcinelli. The indications for this technique are all those types of corneal blindness not treatable by penetrating keratoplasty.

■ 4228

Clinical decision paths in KPro Surgery

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Purpose To analyse the currently available methods for treating very severe anterior segment disease, such as stem cell transplantation with amniotic membrane transplantation, lamellar and penetrating keratoplasty techniques, and the different Kpro's currently available, in regard to the initial clinical findings, the potential complications encountered and the surgical requirements needed for the different techniques. Factors considered are: uni- or bilaterality, limbal stem cell status, dry eye status and availability of healthy teeth.

Methods A systematic analysis of surgical options available for different stages of a variety of anterior segment diseases and currently published results of VA and complications

Results With a systematic approach it becomes clear that some popular reconstructive surgical techniques should be avoided in cases where a very low chance of success is to be expected (e.g. amniotic membrane and stem cell transplantation and/or PKP in very dry eyes → these would have to be treated with OOKP).

Conclusion Following a simple the clinical decision path the anterior segment surgeon will be presented with standardized guidelines for treating those patients where conventional surgical procedures have to be avoided and replaced by rather rarely performed KPro techniques.

■ 4231

Safety evaluation of intravitreal use of a beta2-agonist in rabbit eyes

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Purpose There is no known information on the use and safety of a long-acting beta-agonist, such as Clenbuterol when administered by intravitreal injection. Therefore, it is appropriate to perform this intravitreal injection in an animal model prior to start with a human experiment. The aim is to investigate the safety of an intravitreal injection of the beta2-agonist Clenbuterol in rabbit eyes. This study is in preparation of using of this molecule in human eyes. That trial will be a monocentric, academic (investigator driven) trial to investigate the safety and efficacy of an intravitreal beta2-agonist in patients with persistent subfoveal fluid after retinal detachment surgery. Approval of the ethics committee for the human trial has already been obtained, pending a re-evaluation after the results of the animal study will be known.

Methods 5 rabbits will receive an injection of 0.1 ml solution containing 0.08µg Clenbuterol in one eye and an injection of 0.1ml NaCl 0.9% in the other eye. Since the volume of an adult rabbit eye is only one third of an adult human eye, the achieved concentration will be three times the concentration suggested for use in a human eye.

■ 4233

Can preoperative bevacizumab improve trabeculectomy outcome? Avastin-Trab study

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Purpose The aim of this project is to study whether preoperative intracameral bevacizumab (Avastin) might improve the outcome of filtration surgery.

Methods This study will be carried out in a prospective, placebo-controlled, double-blinded experimental setup. The effect of preoperative administration of bevacizumab on intraocular pressure, bleb characteristics and post-operative medication and surgical intervention will be investigated. The risk of systemic side-effects will be minimized by using local anti-Vascular Endothelial Growth Factor treatment. The study patients will be divided into two major groups: A) Patients with primary open angle glaucoma and B) Patients with normotensive glaucoma, in which very low IOPs are targeted. Both groups of patients will undergo a trabeculectomy. Patients in group A will not be given the antimetabolite Mitomycin C (MMC), while patients in group B will receive MMC to obtain sufficiently low IOPs. This strategy adheres to standard operating procedures for filtration surgery.

Results will follow

Conclusion Our study will potentially shed new light on a plausible and simple method to improve the prognosis of glaucoma filtration surgery. Since this study will provide direct data on the effectiveness of a one-time treatment that might reduce the risk of bleb failure after filtration surgery, avoiding or reducing the need for long-term medication use or secondary surgical intervention, the potential clinical implications of this study are clear. Thus, our project opens exciting new perspectives for the treatment and prognosis of the blinding condition of glaucoma

■ 4232

The role of LOX and LOXL2 in inflammation and fibrosis in a laser induced mouse model

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Purpose Lysyl oxidase (LOX) and lysyl oxidase-like protein 2 (LOXL2) are involved in the cross-linking of collagen and elastin in the extracellular space. The aim of this study was to investigate the expression LOX and LOXL2 in the eye (choroid and retina) after laser-induced chorioidal neovascularization (CNV). We also want to check the anti-angiogenic, anti-inflammatory and anti-fibrotic efficacy of anti-LOX and anti-LOXL2 antibody in a mouse model of age related macular degeneration (ARMD).

Methods CNV will be induced in 8 to 10 weeks old C75Bl/6 mice, by placing 3 laser spots at 9, 12 and 3 o'clock position (50µm, 0.05 s and 400mW). Two daily injections with LOX(L) antibodies or control solution (PBS-Tween 20%) will be given intraperitoneally from day 0 after laser until day 34. Immediately after death, both eyes will be enucleated. One eye will be used to check RNA-expression of LOX and LOXL. The other eye will be used to perform different immunohistochemical stainings (HE, Sirius Red, Trichrome, CD31, CD45 and LOX(L)).

Results Preliminary results showed a significant increase of LOX and LOXL2 in the choroid and retina after laser compared to control.

Conclusion ARMD remains the most common cause of irreversible vision loss in people aged 50 years and older, due to (sub-)retinal neovascularisation and scarring. It is already known that LOX and LOXL are playing a role in the cross linking of collagen and elastin, leading to an increase of fibrosis, and there is growing evidence that these molecules also play a role in neovascularisation. Therefore, a therapeutic potential of anti-LOX and/or anti-LOXL therapy can open new perspectives to treat CNV.

■ 4234

Is neuroglobin (Ngb) a perspective for glaucoma?

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Purpose To further explore the mechanisms of Ngb function in the retina, and investigate the possible neuroprotective potency of this O₂-binding protein in glaucoma. Our project will potentially open exciting perspectives for the treatment and prognosis of the blinding condition of glaucoma.

Methods

1) In vitro: We will set up cultures for primary RGCs. The cultured RGCs will be subjected to hypoxia by incubation in a controlled-atmosphere incubator. Cell survival and apoptosis will be assessed using MTT method and TUNEL staining. Ngb expression in normoxic and hypoxic cells will be compared. Additionally, the RGCs will be transfected with plasmids expressing Ngb mRNA to evaluate the neuroprotective effect of Ngb.

2) In vivo: The ischemia/reperfusion model and the staurosporine intravitreal injection model cause acute RGC loss. We will also use a laser model to induce chronic elevated IOP by obstructing the aqueous outflow pathway. We will first analyze upregulated Ngb expression in these models. The localization of both Ngb mRNA and protein will be assessed. Ngb-Tg mice will be subjected to the glaucoma models. We will measure cell density or retinal thickness and perform electroretinography to evaluate the physiological function of retina. We will also analyze the possible contribution of oxidative stress via immunostainings for ROS and determine possible effects on NO scavenging by evaluating the expression of NOS. Furthermore, we will transfect RGCs in vivo using intravitreal injections of a Ngb expressing adeno-associated virus (AAV) to test the therapeutic potential of Ngb.

Conclusion Our project will not only provide us with an improved understanding of neuroglobin, but also open a new perspective for the treatment of retinal ganglion cell apoptosis and glaucomatous neuropathy.

■ 4235

Immunomodulation of corneal epithelial cells following electroporation with mRNA encoding IL-10 and FasL

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Purpose The aim of this project is to transfect corneal epithelial cells with mRNA encoding IL-10 and FasL in order to evoke down modulation of allogenic T cell responses in an ex vivo model.

Methods The corneal epithelial cells (CECs) will be electroporated initially using a reporter gene, EGFP, in order to optimize the transfection efficiency of the cells which will be detected using flow cytometry. Following this, we intend to transfect the cells with mRNA encoding IL-10 and FasL. The cells will then be labeled with antibodies against IL-10 and FasL and checked for expression of these molecules using flow cytometry. Cytokine secretion will also be detected using ELISA. IL-10 and FasL transfected cells will be co cultured with allogenic T cells and compared with control co cultures of allo-T cells with mock-electroporated CECs. After 5 days of culture, T cells will be analyzed for allogenic reactivity by ELISA for IFN- γ production in the culture supernatant.

Results We intend to show that it is possible to electroporate corneal epithelial cells effectively ex vivo with a reporter gene as well as with IL-10 and FasL. We hypothesize that this may induce tolerance in allogenic T cells and we intend to illustrate this in an ex vivo model using an epithelial-allo T cell co-culture.

Conclusion This project aims to illustrate, in an ex vivo model, how gene insertion into corneal epithelial cells could possibly lead to improved cultivated limbal stem cell graft acceptance in patients with vascularized corneas.

■ 4241

Fundamentals of cataract development

WEGENER A

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The lens is a highly specialized tissue in the vertebrate eye, whose growth characteristics and metabolic turn-over are designed for longevity in respect to its optical function. It grows throughout life and does neither shed any of its cells, nor excrete any degraded proteins. The lens manages to maintain an optical and a biochemical gradient based on the high protein content (crystallins) and slow metabolic turn-over in the superficial fibre cells. It is well equipped with anti-oxidative defence mechanisms, can seal off groups of damaged fibre cells and changes its transmission properties during ageing. The 3 main ageing characteristics in the lens are a tissue layer specific increase in light scattering, a yellowish discoloration of the proteins and a loss of accommodative capacity. Cataract development can be triggered by a wide selection of factors including genetic predisposition, certain diseases, optical and ionizing radiation, chemical compounds, drugs, nutritional and environmental factors, as well as trauma. Patho-physiological mechanisms involved are DNA damage in epithelial cells, protein glycosylation and aggregation and molecular cross linking in lens fibre cells, metabolic breakdown of selected groups of fibre cells or of a whole layer, protein degradation and finally globular degeneration and liquefaction. Ageing in this context is the time line which allows an increasing number of noxious factors to interact in the lens at decreasing metabolic capacities. While the sensitivity of the lens to oxidative damage increases during ageing, its sensitivity to low dose ionizing radiation decreases. The function of molecular receptors for acetylcholine, steroids, the sigma receptor and other receptors found in the lens is still unknown, but might explain the specific reaction to certain drugs.

■ 4243

MICS, mastering the critical steps

BELLUCCI R

Verona

Although every manoeuvre has to be modified for MICS, there are some more critical steps. Capsulorhexis through 1.8 mm incision requires a dedicated forceps, usually coaxial, that should be appropriately managed. However it is easier than capsulorhexis through larger incision, because of the improved visibility and chamber stability. The fluidics control of MICS is also different from that of standard phacoemulsification, and includes low hysteresis of the system, reduced fluid volume, dual control of vacuum and ultrasound, and the removal of lens particles from the aspiration line. With this "microfluidics" of phacoemulsification there is no need to increase the bottle height above 90 cm.

■ 4242

Micro-incision (MICS), perfecting wound incision

COCHENER B

University Hospital Morvan, Dept. of Ophthalmology, Brest

It is nowadays well admitted that a perfect incision in microincision (means size inferior to 2 mm) is crucial: for easy manipulation in the eye, anterior chamber stability during surgery, self sealing without suture and no induced astigmatism. This presentation will focus on incision architecture including location, size, shape and self sealing. We will provide some practical tips and tricks for performing a corneal incision as perfect as possible, which is the key for a successful MICS, whatever a bimanual (B-MICS) or coaxial approach (C-MICS).

■ 4244

MICS and vitreo retinal surgeries

CREUZOT C

Department of Ophthalmology - University Hospital, Dijon

Purpose Cataract represents the main complication occurring after vitreoretinal surgery. The purpose is to present the main advantages and conveniences due to combined procedures.

Methods Among the complications of vitrectomy, cataract represents the most common one as about 100% of patients will be operated within 5 years after initial surgery. However, surgeons have to face sometimes with severe complications when operating patient long time after vitreoretinal surgery. Combined procedures have become more and more popular especially for macular surgery.

Results The main pros and cons of combined surgery have to be considered: specific surgical complications, problems due to the internal tamponade, macular edema, cost... Combined procedures will probably become the standard procedure in vitreoretinal surgeries. The use of transconjunctival vitrectomy as well as MICS contributes to the easier post-operative follow-up of the patients.

Conclusion Combined surgery for vitreoretinal diseases will probably become the standard procedure due to the diffusion of transconjunctival surgery.

■ 4245

MICS and glaucoma

BRON AM

Ophthalmology, University Hospital, Dijon

Purpose Anterior segment imaging devices have brought a better understanding of the role of the lens in angle-closure glaucoma. The key-role of the lens in these presentations seems to be crucial in open-angle glaucoma as well.

Methods With the growing evidence that the lens plays a more important role in glaucoma than previously thought, it is expected that lens extraction will increase in the next coming years. Micro incision cataract surgery (MICS) offers a unique opportunity to achieve for these cases a safe and reliable procedure. We will focus on combined surgeries with lens extraction (either trabeculectomy or deep sclerectomy). The small size of the corneal incision allows a very good tightness of the incision. Therefore glaucoma surgery is performed on a hard globe with much ease.

Conclusion MICS although dedicated to the field of cataract, yields a valuable improvement in the surgical management of the glaucomas.

■ 4251

Prophylaxis of posttraumatic endophthalmitis

ABU EL ASRARA

King Abdulaziz University Hospital, Ophthalmology, Riyadh

Infectious endophthalmitis is a devastating complication of open globe injuries. The incidence of culture-positive endophthalmitis after open globe injuries varies between 0.5% and 17%. Several reports have demonstrated that delayed primary repair, dirty wound, breach of lens capsule, retained intraocular foreign body (IOFB), grade 4 injury (presenting visual acuity of worse than 5/200 to light perception), placement of primary intraocular lens, and rural setting are associated with an increased risk of posttraumatic endophthalmitis. Posttraumatic endophthalmitis is associated with its own microbiologic spectrum which is distinct from other subgroups of exogenous endophthalmitis. Posttraumatic endophthalmitis still carries a poor prognosis. Reasons for guarded prognosis include polymicrobial infection and the virulence of the infecting microorganisms. In addition, concomitant injuries may directly result in ocular damage that limits ultimate visual recovery. Because of the substantial incidence of endophthalmitis after open globe injuries, careful consideration should be given to the use of prophylactic antimicrobial therapy. The purpose of prophylaxis is to provide effective antibiotic levels as rapidly as possible against a broad range of organisms. Good coverage for most organisms is obtained with intravenous vancomycin coupled with a third generation cephalosporin, such as ceftazidime, which can penetrate the vitreous cavity in effective levels in inflamed aphakic experimental eyes. Recently, the use of prophylactic intravitreal antibiotic administration in high-risk cases was recommended.

■ 4253

Bacterial keratitis: the clinical problem

PLEYER U

Berlin

ABSTRACT NOT PROVIDED

■ 4252

Animal studies for the prevention of endophthalmitis

KOWALSKI RP

Pittsburgh

Topical anti-infectives are commonly used to prevent post-surgical endophthalmitis. Animal models can be used for the development and selection of topical anti-infectives for optimizing ocular surgical prophylaxis. The primary outcome measures for providing prophylactic efficiency are antibacterial efficacy and anti-infective penetration into the anterior chamber. This current presentation will focus on a rabbit prevention of endophthalmitis model. Publish data will be presented detailing clinical correlation and limitations of the models.

■ 4254

Animal models for the treatment of bacterial keratitis

KOWALSKI RP

Pittsburgh

Rabbit models of bacterial keratitis have been used to evaluate the efficacy of anti-infectives in the clinical treatment of bacterial keratitis. These models can determine:

1. ocular toxicity and tolerance of anti-infectives to ocular tissue,
2. penetration of anti-infectives into the cornea, and
3. anti-bacterial efficacy of the anti-infectives to corneal bacterial pathogens.

The current presentation will cover the structure and limitations of rabbit bacterial keratitis modeling using published data. Topics will include statistical design, the choice of bacterial pathogens, and positive aspects for possible systemic anti-infective development.

■ 4255

The use of animal models for the evaluation of ocular antiviral agents

ROMANOWSKI EG

The Charles T. Campbell Laboratory, UPMC Eye Center, Ophthalmology and Visual Sciences Research Center, Eye and Ear Institute, Department of Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh

Animal models have been used extensively in the evaluation and development of topical ocular antiviral agents. Rabbit ocular models have proven predictive of the clinical efficacy of topical antiviral agents for HSV-1 epithelial keratitis and in clinical trials for adenoviral ocular infections. The current presentation will discuss the limitations of these models and present data on potential new topical antiviral agents, focusing primarily on antivirals for adenoviral ocular infections.

■ 4261

Basic principles of radiotherapy in ophthalmic oncology

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 (2) Ophthalmology, Paris

Ophthalmologic Radiation Oncology concerns intra ocular tumors, orbital tumors and eyelid tumors. In these locations, tumors are often close to normal critical tissues, increasing the risk of radiation toxicities. Basic principles of ophthalmic radiotherapy are not really different from radiotherapy of others organs. They are based on a precise description of the tumor volume, knowledge of potential tumor local or regional extension, knowledge of tissue biological radiosensitivity for tumors and the surrounding normal tissues. Based on these features, treatment modalities (radiation treatment length and fractionation) are determined to fulfill the aims of medical curative intents: good local control with acceptable toxicities or the aims of medical palliative intents: to relief with minimal toxicities. Many types of radiation therapy can be used for ophthalmic tumors: brachytherapy, orthovoltage radiotherapy, conformal therapy with photons and electrons including intensity modulated radiation therapy, protontherapy, and the use of specific devices such as CyberKnife and Tomotherapy. All of these techniques have their advantages and their disadvantages, leading the radiation oncologist to make a choice depending on the tumor site and characteristics.

■ 4263

18 years experience of the use of proton beam in ophthalmic tumours

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 (1) Ophthalmology, Paris
 (2) Biostatistics, Paris
 (3) Medical Physics, Orsay
 (4) Radiotherapy, Paris

Purpose Since 1991, more than 3000 patients were treated at Curie Institute by proton beam for uveal melanoma. All datas concerning tumor characteristics, treatment planning and follow up are registered in the data base

Methods We have performed a retrospective review of patients treated before 2005. Patients were sent to Curie by their local ophthalmologist. Clip positioning and proton beam therapy with a dose of 60 grays in 4 fractions was scheduled; All patients were seen once a year in our clinic after treatment with liver ultrasounds twice a year.

Results 2214 patients were treated, median age 61 years, median diameter 13,6 mm, median Thickness 4,7 mm. 20% of patients developed metastasis, 25% of patients died, 4% had local recurrences, 6,7% had secondary enucleations; With multivariate analysis Significant risk factors for metastasis were age (>60), tumor location, tumor diameter and tumor thickness (p<0,0001); Significant risk factor for local recurrence was tumor diameter (p<0,001) significant risk factor for secondary enucleation were tumor diameter (p<0,0001) and tumor thickness (p= 0,003) Neovascular glaucoma was 10% at 2 years , 30% at 5 years and 34% at 10 years. Maculopathy was 40% at 2 years, 62% at 5 years and 66% at 10 years. Optic neuropathy was 14% at 2 years, 29% at 5 years and 38% at 10 years At last follow up 36,2% of patients have more than 20/40 vision

Conclusion Tumor control obtained by proton beam is excellent with a long follow up For big tumors new additional techniques like endoresections, additional TTT or anti VEGF could enhance globe preservation

■ 4262

The treatment of uveal melanoma by ruthenium plaques

DAMATO B
 Liverpool

Purpose Plaque radiotherapy is the first choice of treatment for choroidal melanomas. The standard practice is to position the plaque centrally in relation to the tumour, with a 2mm physical safety margin in all directions. The author has developed techniques for administering brachytherapy with plaques positioned eccentrically, with their posterior edge aligned with the posterior tumour margin. This allows a higher apex dose to be given while reducing the radiation delivered to optic nerve and fovea.

Methods The tumour is localized by transillumination and its anterior margin marked on the sclera with a pen. The 'plaque-tumour difference' measurement is then marked on the sclera at the point where the anterior plaque margin should be. A transparent plaque template is sutured to the sclera. The position of the plaque is checked with a right-angled fibre-optic transilluminator, which is passed through a perforation near the posterior edge of the template. If the template is positioned correctly, then with binocular indirect ophthalmoscopy one should see the 'sunset sign' which occurs when the transilluminator is exactly at the posterior tumour margin.

Results Eccentric ruthenium plaque positioning has been practised in Liverpool for over a decade. The results have been published. Local tumour control rates have been good (partly because of case selection). Radiation maculopathy and optic neuropathy have been rare if the tumour has not extended within 1 mm of these structures. The main cause of visual loss has been maculopathy, caused by exudation from the irradiated tumour. Methods for treating such exudation are described.

Conclusion Plaque radiotherapy is effective for choroidal melanomas up to 5 mm in thickness. It achieves good local tumour control with preservation of vision in most cases.

■ 4264

Limiting the sequelae of irradiation in retinoblastoma patients by using globe and orbital brachytherapy with iodine 125

STANNARD C

ABSTRACT NOT PROVIDED

■ 4265

The use of ruthenium plaque brachytherapy in retinoblastoma

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Purpose To evaluate the efficacy of 106Ru plaque brachytherapy for the treatment of retinoblastoma.

Methods We reviewed a retrospective, noncomparative case series of 39 children with retinoblastoma treated with 106Ru plaques at the Jules-Gonin Eye Hospital between October 1992 and July 2006, with 12 months of follow-up.

Results A total of 63 tumors were treated with 106Ru brachytherapy in 41 eyes. The median patient age was 27 months. 106Ru brachytherapy was the first-line treatment for 3 tumors (4.8%), second-line treatment for 13 (20.6%), and salvage treatment for 47 tumors (74.6%) resistant to other treatment modalities. Overall tumor control was achieved in 73% at 1 year. Tumor recurrence at 12 months was observed in 2 (12.5%) of 16 tumors for which 106Ru brachytherapy was used as the first- or second-line treatment and in 15 (31.9%) of 47 tumors for which 106Ru brachytherapy was used as salvage treatment. Eye retention was achieved in 76% of cases (31 of 41 eyes). Univariate and multivariate analyses revealed no statistically significant risk factors for tumor recurrence. Radiation complications included retinal detachment in 7 (17.1%), proliferative retinopathy in 1 (2.4%), and subcapsular cataract in 4 (9.7%) of 41 eyes.

Conclusion 106Ru brachytherapy is an effective treatment for retinoblastoma, with few secondary complications. Local vitreous seeding can be successfully treated with 106Ru brachytherapy.

■ 4266

The treatment of uveal melanoma with iodine plaque brachytherapy

KIVELÄ T
Helsinki University Central Hospital, Helsinki

Purpose To provide an overview of managing uveal melanoma (UM) with iodine brachytherapy (IBT).

Methods Personal experience of the author in using IBT since 1990.

Results IBT is an effective option for managing a UM of any size, although it is mostly used for medium-sized tumours, preference being given to ruthenium brachytherapy (RBT) when the tumour is <5-6 mm thick and to transscleral local resection when thickness is >6 mm, especially when vision is good. IBT is also a safe alternative to enucleation of large UM >10 mm in thickness if the patient is keen to preserve the eye and motivated to accept eventual complications. The plaque is positioned over the UM with a 1-2 mm safety margin when using a collimated/rimmed plaque. Because of stray radiation, a safety margin is not mandatory when the plaque is non-collimated/non-rimmed. Otherwise, surgical technique does not differ from RBT. An advantage of IBT is that the radioactive seeds are separate from the plaque, allowing economical use of plaques of many different sizes and shapes and individual positioning of the seeds in a conformal way. A disadvantage is a short half-life; the seeds need to be changed every 6 months. The dose the author uses is 80 Gy to tumour apex, which is reduced on an individual basis to 60-70 Gy when the UM is very thick. Local tumour control rate is 90% and, paradoxically, not worse for large UM as compared to smaller ones. There are no unequivocal safety distances for avoiding radiation cataract, maculopathy and optic neuropathy, which are more or less frequent depending on the size and location of the UM.

Conclusion IBT achieves good local tumour control of UM of all sizes, but preservation of vision is decidedly less frequent than after RBT, which is always given preference.

■ 4311

Principle of adaptive optics

SÖDERBERG PG

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Purpose To provide an overview of adaptive optics imaging of the retina.

Methods In ophthalmoscopic imaging, the two dimensional spatial radiance variation of the back scattered light from the retinal surface is measured. Perfect imaging would require that light backscattered from one point on the retina examined is refocused to one point on the detector. Due to diffraction, light scattering and aberrations, some of the light, injected into the eye examined and some of the light back scattered from the retina examined, are deviated. This leads to loss of contrast. Aberrations induced are, for each individual, specific to the optics of the eye examined. In AOSLO, in addition to confocal illumination and light detection, aberrations induced by the optics of the eye examined are individually measured by wave front sensing and corrected for.

Results In the AOSLO, a wave front sensor is introduced between the light source and the eye examined. The information from the wave front sensor is fed back to a deformable mirror, also placed in between the light source and the eye examined. The deformable mirror corrects the wave front aberrations induced by the optics of the eye examined. This allows the injected light to form a point on the retinal surface and simultaneously, the backscattered light from the retina of the eye examined to be focused to a point on a detector. An x-y scanner in front of the eye allows sequential illumination and capturing of aberration minimized back scattered light from an x-y matrix of the retinal surface of the eye examined. The relative radiances measured in the x-y matrix represent the image.

Conclusion Adaptive optics in ophthalmoscopy improves contrast in the image of the retina examined by correcting for aberrations induced by the optics of the eye examined.

■ 4313

Spectral domain OCT of exudative AMD

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Creteil Eye University, Creteil

Age-related Macular Degeneration (AMD) is the main cause of vision loss in developed countries. Spectral domain OCT (SD-OCT) is a non invasive technique providing in vivo imaging of the retina, with a higher resolution than time domain OCT. This SIS will describe the clinical features of exudative AMD with SD-OCT, including occult choroidal neovascularization (CNV), classic CNV, idiopathic polypoidal vasculopathy, and chorioretinal anastomosis. The improvement of the resolution of retinal imaging will provide a better classification and explanation of the pathological processes observed during AMD.

■ 4312

Telangiectasia evaluated with adaptive optics and HR-OCT

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Centre Hospitalier Intercommunal de Creteil, Creteil

Purpose Type 2 Macular Telangiectasia is a progressive disease starting in the fifth to seventh decade and characterized by a progressive damage of the neurosensory retina.. The purpose of this study is to compare the images obtained using two non-invasive techniques, High-Resolution Optical Coherence Tomography (HR-OCT) and Adaptive Optics (AO), in Type 2 Macular Telangiectasia.

Methods Nine eyes of 5 patients affected by Type 2 Macular Telangiectasia underwent examination including visual acuity measurement with ETDRS (Early Treatment of Diabetic Retinopathy Study) chart, color photographs, monochromatic photographs, Spectral-Domain Optical Coherence Tomography with Heidelberg Spectralis "OCT and Adaptive Optics assessment with Imagine Eyes" System. The neurosensory retina and the photoreceptor layer were analyzed using both HR-OCT and AO imaging.

Results The disruptions of the photoreceptor layer on HR-OCT correspond to a cellular loss on Adaptive Optics Imaging. On the other hand, Adaptive optics Imaging allows the measurement of cellular density in areas which have a normal aspect on HR-OCT.

Conclusion The diagnosis of Macular Telangiectasia is based on biomicroscopy and fluorescein angiography. Adaptive Optics Imaging is helpful to analyze the retinal damages, especially the cone abnormalities. This technique will certainly allow a better understanding of this rare disease. Author Disclosure Information: K. Atmani, None; N. Leveziel, None; G. Soubrane, None.

■ 4314

Drusen in adaptive optics and SD-OCT

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(2) *Imagine Eyes, Orsay*

Purpose The study objective was to explore the microscopic structure of soft macular drusen and surrounding retinal areas using an adaptive optics (AO) camera and to compare the findings to those from standard clinical examinations.

Methods 18 patients of age between 65 and 85 and presenting soft macular drusen were recruited after an initial scanning laser ophthalmoscope (SLO) examination. We used an AO flood-illumination system to acquire high-resolution images of selected drusen areas. Every acquisition provided a series of 20 consecutive reflectance images, out of which 10 were numerically averaged to produce an enhanced final image. The resulting AO images were analyzed in comparison with conventional infrared and autofluorescence fundus images and spectral optical coherence tomography scans.

Results The soft drusen were visible in AO images as generally round areas delimited by a peripheral low-reflectance line. Hyper reflective spots of size comprised between 2 and 15 µm were observed in many drusen inner areas. These bright spots were sometimes isolated, sometimes grouped into tight aggregates of 2 to 40 components. Cone photoreceptors were visible in areas between drusen in most AO images.

Conclusion The microscopic structures observed in the AO images of soft drusen presents analogies with their described anatomopathologic characteristics, which could not be identified using other in vivo imaging techniques. AO technology could help to refine the clinical classification of macular drusen and obtain deeper insight in their link with the development of different types of advanced AMD. Author Disclosure Information: N. Massamba, None; B. Lamory, Imagine Eyes, G. Soubrane, None.

■ 4315

Chorioretinal anastomosis in adaptive optic and high definition spectral domain optical coherence tomography

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Purpose To assess morphologic variations in the outer and inner retinal layers in eyes with chorioretinal anastomosis using high definition Spectral Domain Optical Coherence Tomography (Spectralis HRA OCT, Heidelberg Engineering, Heidelberg, Germany) and to compare these scans with images acquired by Adaptive Optics (AO).

Methods This was a prospective observational case series including 50 patients. SD-OCT scans were obtained with combined confocal scanning laser ophthalmoscope (cSLO) and SD-OCT for simultaneous tomographic and topographic in vivo imaging. Patients underwent fluorescein and ICG- angiography and Adaptive Optics assessment with Imagine Eyes™ System. The neurosensory retina and the photoreceptor layer were analyzed using both HR-OCT and AO imaging.

Results Combining of the adaptive optics with SD-OCT may give us further information of the early stage development of chorioretinal anastomosis.

■ 4316

Perspective for adaptive optics

CHATEAUN, LAMORY B
Orsay

ABSTRACT NOT PROVIDED

■ 4321

Runnig a clinical service for KPros

BARATZ K

ABSTRACT NOT PROVIDED

■ 4322

How do we get started with offering MOOKP clinical service?

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Modified osteo-odonto keratoprosthesis (MOOKP) is complicated two step surgery. Firstly, we must understand why it is effective for visual recovery of end-stage ocular surface diseases like Stevens- Johnson syndrome. MOOKP have a lot of advantages compared to other K-pros, for example the using auto tissue of canine tooth root and buccal mucous membrane, the tight adhesions between optical cylinder and canine tooth root, the adhesion between MOOKP lamina and sclera or cornea, the strong ocular surface by auto buccal mucous membrane, no inflammation on the back of optical cylinder and so on. However, the precise surgical techniques and proper instructions are necessary to succeed the very first case in newly set surgical center. In Japan, we successfully set up the MOOKP center and did perform 4 cases of MOOKP since 2003. We share our experience about it and point out our modification adjustable for Japanese patients.

■ 4323

Why we never use a KPro and MOOKP

KINOSHITA SS

Kyoto

ABSTRACT NOT PROVIDED

■ 4324

Inflammatory responses to KPro: can we control them?

PEREZ VS

Miami

ABSTRACT NOT PROVIDED

■ 4325

Retinal detachment in Falcinelli's modified osteodontokeratoprosthesis

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(2) Osteodontokeratoprosthesis Foundation, Rome

Purpose Aim is to evaluate the incidence, surgical treatment and outcomes of retinal detachment in eyes that had undergone Falcinelli's modified osteodontokeratoprosthesis (MOOKP).

Methods Technological and surgical advancements allow to treat successfully a severe pathology as retinal detachment, even in eyes with keratoprosthesis (KPro). The authors accurately describe the surgical technique which usually uses an Eckardt or Landers temporary KPro, a pars plana vitrectomy and a gas or silicone oil tamponade, even if in selected cases it is possible to perform just a scleral buckling.

Results By the means of the described techniques good anatomical success and improvement in visual acuity have been obtained. Nine retinal detachments were successfully operated, one retinal detachment was unsuccessfully operated, four retinal detachments were judged to be inoperable for severe proliferative vitreoretinopathy because of late turning-up to clinical examination and lack of technology in the 70's.

Conclusion The authors point out that an accurate MOOKP procedure it is necessary for preventing the retinal detachment. An early diagnosis by echography performed at every clinical examination during the follow-up in patients with MOOKP and an appropriate surgical planning for each case are fundamental for a better anatomic-functional outcome.

■ 4327

Management of oculoplastic problems in the OOKP eye

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OOKP surgery (either stage 1 or 2) can result in complex oculoplastic complications. The majority of these are mucous membrane graft-related, including graft thinning, ulceration and infection or overgrowth onto the anterior optical cylinder. However, lid malposition, forniceal shortening and widening of the palpebral aperture may also occur. Appropriate timely surgical intervention of these is crucial to protect the underlying OOKP lamina. In addition, correcting over-exposure of the globe aids in improved comfort for the patient. Adjustments of the lid and forniceal abnormalities can enhance comfort, cosmesis and prosthetic shell stability. Retrospective case note analysis of all patients treated 1996 – 2009 at the Sussex Eye Hospital, Brighton, UK, was performed. This study reports the prevalence and type of oculoplastic complications found and describes the surgical management for each.

■ 4326

Tibial Bone KPro technique and long term results

TEMPRANO J
Barcelona

The operation is performed in three stages. The first stage consists in preparing the eye to receive and maintain the keratoprosthesis. For this purpose the anterior surface of the eye is cleaned and regularized, eliminating fibrous tissue and the entire epithelium. Subsequently we obtain a 2 x 3 cm graft of buccal mucosa from the inferior lip comprising the entire mucosal and submucosal thickness. The graft is sutured to cover the anterior pole of the eye to promote revitalization. The second stage consists in preparing the keratoprosthesis. A 10 mm disk of tibial bone from the superior part of the medial face of the tibia is obtained using a crown drill. The posterior part of the piece of bone obtained is then cut with a chisel to obtain a thickness of 3 mm. Subsequently the obtained disk of bone is cleaned and a central opening of 3.5 mm is performed to introduce in this opening a PMMA optic cylinder; 9 mm in length, 3.5 mm in diameter in its narrow portion, 4 mm in the wider portion. Fixation is achieved with cyanoacrylate. This is left to dry and then it is introduced into a palpebral pocket of the inferior lid of the patient. The pocket is closed with sutures and the piece is left in place for three months. For the third stage we remove the keratoprosthesis device from the palpebral pocket and if it is found to be in perfect conditions we dissect the buccal mucous membrane which is covering the cornea and perform a central window with a 4.5 mm trephine to remove the transparent or cataractous lens and perform a total iridectomy. The posterior portion of the optic cylinder is introduced into the anterior chamber. The prosthesis is sutured to the anterior pole of the eye with non-absorbible sutures. Finally the buccal mucosa is replaced, covering the entire area. One point of blepharorrhaphy is applied. Long term results. We started to use this technique in 1988 and after 21 years of experience we have 80% of anatomically perfect results. In 20 % of the cases the prosthesis extruded due to total or partial resorption of the bone. It has to be emphasized that these were cases without any other possibility of treatment. We did 143 cases during these years. The longest follow-up of a prosthesis "in situ" is 19 years. The earliest extrusion was after one year. The complications are the same as for OOKP (glaucoma, retinal detachment, vitritis, extrusion) The functional results depend on the conditions of the retina and the optic nerve. There were many cases with 20/20 vision. The mean value of retention of the prosthesis is 15 years.

■ 4328

Dealing with complications of MOOKP: VR

HUGHES E
Bristol

ABSTRACT NOT PROVIDED

■ 4331

Quantification of neuronal cells in healthy rat retinas by flow cytometry

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Purpose The primary purpose of this study was to evaluate the potential to quantify the different neuronal populations in the retina of healthy Sprague–Dawley rats in an accurate quantitative way by using flow cytometry.

Methods Rats were killed and the eyes were enucleated to achieve retinal dissection. Tissue dissociation was accomplished with trypsin. After trypsin action was blocked the cells were mechanically dissociated into a single-cell suspension by gentle pipetting. The cells were permeabilized and stained with the primary antibody. This incubation was followed by another one with a blocking antibody and finally the cells were incubated with the secondary antibody. At least 100.000 healthy cells were analyzed with a FACScalibur and FlowJo software. Dead cell and cell debris were excluded from analysis by gating with forward scatter and side scatter as indicators. The primary antibodies were anti-rhodopsin against photoreceptors, anti-Protein Kinase C against bipolar cells, anti-calbindin against horizontal cells and anti-microtubule associated protein 1 against ganglion cells. All the primary antibodies were monoclonal and the secondary antibody was goat anti-mouse Alexa Fluor 594.

Results Quantification of the above populations was possible using flow cytometry. In this preliminary study, the photoreceptors had the 53.99%, the ganglion the 7%, the bipolars the 4% and the horizontal the 1.15% in the whole mixed retinal population. All experiments were repeated six times.

Conclusion Flow cytometry can be used to quantify the different neuronal populations in control healthy eyes and this verification will be very useful in future in studies in apoptosis or proliferation of these cells.

■ 4333

Role of HSP70 and p62 in regulation of autophagy clearance in ARPE-19 cells

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Purpose A hallmark of RPE cell aging is lysosomal lipofuscin accumulation reflecting a weakened capacity for protein degradation in lysosomes. The presence of lipofuscin increases the misfolding of intracellular proteins, which evokes additional stress in the RPE cells. If the capacity of molecular chaperones to repair protein damages is overwhelmed, then the proteins are mainly cleared in proteasomes or in lysosomes including autophagy. We demonstrate that autophagy is a master clearance mechanism in proteasome inhibitor -induced aggregation in ARPE-19 cells.

Methods The HSP70, p62 and ubiquitin expression levels and localization were analyzed by western blotting and immunofluorescence. Confocal and transmission electron microscopy were used to detect cellular organelles and to evaluate morphological changes. HSP70 and p62 levels were modulated using RNA interference and overexpression techniques. Cell viability was measured by colorimetric assay.

Results The proteasome inhibitor MG132 evoked the formation of juxtannuclear protein aggregates in ARPE-19 cells. It also caused a robust accumulation of HSP70 and p62 proteins and ubiquitin-protein conjugates that all colocalized with the formed protein aggregates. We found that protein aggregation is a temporary process, a cessation of proteasome inhibition led to autophagosome-mediated removal of cytoplasmic protein aggregates. Interestingly, the p62 rather than the HSP70 regulate autophagy activity. However, both of them have essential function in regulation of cellular viability.

Conclusion In addition to classical lysosomal proteolysis, there is the increasing evidence that heat shock proteins, proteasomes and autophagy regulate protein turnover in the RPE cells and thus have important roles in AMD disease.

■ 4332

Clearance of dying cells in the retina - relevance to age-related macula degeneration

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Purpose The retina is the place with the highest daily phagocytic turnover in the whole human body. Besides photoreceptor outer segments, retinal pigment epithelial (RPE) cells can engulf other dying cells as well (epithelial, neural, etc.). Failure to do so may result in accumulation of debris that could lead to development of age-related macula degeneration (AMD). The in vitro dynamics of this clearance process can be modelled using human ARPE-19 cells and macrophages.

Methods Different death patterns were induced in vitro in ARPE-19 cells: death through detachment from the extracellular matrix on polyHEMA coated surfaces known as anoikis, UV induced apoptosis and Argon-laser induced necrosis. Two-colored phagocytic assays were carried out where different phagocytes (living ARPE-19 cells or human macrophages) engulfed different dying cells. Flow cytometry (FACS Calibur), fluorescent and time-lapse microscopy (the later not shown) were used to quantify and visualize the phagocytic process.

Results The clearance of the anoikic ARPE-19 cells by the living ARPE-19 cells (serving as a model for dry AMD) over 8 hours of co-incubation proved efficient and increasing over time (at 8 hours, over 50% of the phagocytes contained engulfed anoikic ARPE-19 cells inside). The human macrophages could also engulf the anoikic ARPE-19 cells (serving as a model for wet AMD), although less efficiently at five times lower rate over the same period.

Conclusion The clearance of the different dying ARPE-19 cells can serve as a good in vitro model for studying AMD, both dry and wet type, as well as for testing different immunological and pharmacological aspects affecting this process.

■ 4334

Occludin changes in retinal vessels after experimental vein thrombosis in rats

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Purpose To evaluate the changes in occludin after experimental vein thrombosis in rats. Occludin is an integral plasma-membrane protein located specifically at tight junctions.

Methods The retinal veins of 24 pigmented rats were occluded by means of fluorescein augmented laser photocoagulation. The animals were sacrificed after 4 and 24 hours, their eyes were enucleated and retinas were harvested. Retina whole mounts were prepared for indirect immunohistochemistry using an anti-occludin primary antibody and were examined under confocal microscope. The retinas of 4 normal animals were used as controls.

Results The characteristic reticular fluorescence of occludin was detected along vascular branches in a three-dimensional manner with the help of the confocal microscope. Alterations in occludin distribution could be detected along thrombosed veins, peripherally to the site of thrombosis. These alterations included irregular linear fluorescence and increased spaces of absent fluorescence in major vein branches compared to controls. The alterations in the superficial and deep vascular plexuses were less prominent including a reduced fluorescence of occludin. No changes were detected at the arteriolar walls. All findings were more prominent at 24 hours.

Conclusion Blood-retina barrier impairment is expressed with morphological alterations of occludin distribution 24 hours after experimentally induced vein thrombosis

■ 4335

Structural consequences after intravitreal bevacizumab injection in a retinopathy of prematurity mouse model

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Purpose To evaluate the effect of different bevacizumab concentrations on retinal endothelial cell proliferation and retinal structures in a retinopathy of prematurity (ROP) mouse-model

Methods A total of 35 of C57BL/6 mice were exposed to 75±2% oxygen from postnatal day 7 to postnatal day 12. On day 12, 10 mice (group-C) were injected with 2.5µg intravitreal bevacizumab (IVB), 11 mice (group-D) with 1.25µg IVB, and 14 mice (group-E) with 0.625µg IVB in one eye. The contralateral eyes were injected with isotonic saline (control group=group-B). Four nonexposed mice served as negative controls (group-A). Neovascularization was quantified by counting the endothelial cell nuclei on the vitreal side of the inner limiting membrane of the retina. Histologic and ultrastructural changes were examined by light and electron microscopy.

Results The endothelial cell count per histological section was lower in group C (p<0.0001), D (p<0.0001) and E (p<0.0001) compared with the control group-B. Histologic evaluation showed no retinal toxicity in any group. Electron microscopy revealed hyperoxia induced mitochondrial dysmorphology in group-B and mitochondrial dysmorphology displayed dose dependent increase in IVB injected eyes.

Conclusion Bevacizumab suppresses endothelial cell proliferation in a ROP-mouse-model. In addition to hyperoxia induced vulnerability of C57BL/6 retina, morphological findings implicate further ultrastructural vulnerability due to bevacizumab.

■ 4336

Relationship between VEGF expression and the retina following light-damage

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Purpose In the retina, the balance between pro- and anti-angiogenic factors is critical for angiogenesis and is also involved in cell survival and maintenance. We previously reported an upregulation of VEGF following light-damage (LD). Furthermore, systemic delivery of PEDF, as well as lentiviral gene transfer of an anti-VEGF antibody rescued photoreceptors from cell death. Thus, we investigated the effect of high levels of VEGF on the neural retina and retinal pigmented epithelium (RPE) after LD.

Methods To study the action of VEGF in the retina after LD, we exposed adult Balb/c mice to 5000 lux for 1h, and we collected neural retinas and eye-cups (containing RPE) at different time points after LD. We analysed protein expression, RPE cell permeability and then, we studied the action of VEGF and oxidative stress on ARPE19 cells.

Results In the neural retina, results indicate that high levels of VEGF induce an upregulation of VEGF-R2 and increase the src phosphorylation, whereas VEGF-R1 expression is decreased. Concomitantly with VEGF upregulation, in the RPE we observed a downregulation of PEDF expression and the β-catenin translocates into the cytoplasm of RPE cells, indicating an increase on permeability. We observe that VEGF treatment did not alter ARPE19 cell junctions in normal conditions, whereas under oxidative stress, VEGF contributes to the disruption of cells junctions.

Conclusion This study involves VEGF in LD and highlights the prime importance of angiogenic factor balance for PR survival. Our results suggest that PR apoptosis is augmented by RPE cell permeability, and could contribute indirectly to the deleterious effect of VEGF observed during light-induced PR apoptosis.

■ 4351

Circulating bone marrow-derived endothelial precursor cells contribute to neovascularization in diabetic epiretinal membranes

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Purpose Role of vasculogenesis, recruitment and differentiation of circulating bone marrow-derived endothelial precursor cells into mature endothelium, in proliferative diabetic retinopathy (PDR) remains undefined. We investigated the presence of bone marrow-derived endothelial precursor cells and the expression of the chemotactic pathway SDF-1/CXCL12-CXCR4 in PDR epiretinal membranes.

Methods Membranes from 8 patients with active PDR and 9 patients with inactive PDR were studied by immunohistochemistry using antibodies against CD133, vascular endothelial growth factor receptor-2 (VEGFR-2), CD14, SDF-1 and CXCR4.

Results Blood vessels expressed CD133, VEGFR-2, CD14, SDF-1 and CXCR4 in 10, 10, 10, 7 and 7 out of 17 membranes, respectively. There were significant correlations between number of blood vessels expressing CD34 and number of blood vessels expressing CD133 ($rs=0.646$; $p=0.005$), VEGFR-2 ($rs=0.704$; $p=0.002$), CD14 ($rs=0.564$; $p=0.018$), and SDF-1 ($rs=0.577$; $p=0.015$). Stromal cells in close association with blood vessels expressed CD133, VEGFR-2, CD14, and CXCR4 in 10, 12, 13, and 14 membranes, respectively. Number of blood vessels expressing CD133 ($p=0.013$), VEGFR-2 ($p=0.005$), CD14 ($p=0.008$) and SDF-1 ($p=0.005$), and stromal cells expressing CD133 ($p=0.003$), VEGFR-2 ($p=0.013$) and CD14 ($p=0.002$) was significantly higher in active membranes than in inactive membranes.

Conclusion Bone marrow-derived CD133+ endothelial progenitor cells and CD14+ monocytes may contribute to vasculogenesis in PDR.

■ 4353

Experience with adalimumab for the treatment of non-infectious uveitis

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Purpose Objective: The aim of this study has been to assess the efficacy of adalimumab (ADA) in patients with uveitis in 3 centers.

Methods In a retrospective study we identified patients from all institutions' databases, who were treated with ADA in an average period of 20.16 months (range 1.3-45 months). The 5 criteria that the efficacy of ADA had been judged on are: reduction of macular edema by OCT, visual acuity, anterior chamber cells, reduction of flares and a reduction of prednisone dose during the treatment. At least one of the criteria had to be improved and none of the others worsened to declare treatment as effective.

Results Of the 61 patients who were treated with ADA, 38 were treated for uveitis and systemic disease, 3 primarily for active systemic disease and 20 primarily for active uveitis. 15 patients had been treated before with etanercept and 11 with infliximab, with insufficient response. We saw an improvement in 49 out of these 61 patients (80.3%) in 1 or more criteria and worsening in none, 5 patients did not meet improvement criteria and were given additional or alternative treatment, three of them mainly due to activity of systemic disease. 14 out of 49 (28.6%) patients showed an improvement in every criteria, the other patients showed increasing in at least one criteria, the other criterias remained stable. At the last follow-up there were 50 (81.9%) patients still on ADA treatment. 11 patients stopped ADA treatment for different reasons (inefficacy, active systemic disease, pregnancy etc.).

Conclusion In this retrospective study we judged that the treatment with ADA in patients with uveitis with or without systemic disease was effective in 80.3%. Clinical trials are warranted

Commercial interest

■ 4352

Encouraging results of a VEGF kinoid against experimental choroidal neovascularization

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Purpose The goal of this preliminary study was to evaluate the preventive and curative antiangiogenic properties of a VEGF KINOID vaccine produced by NEOVACS against experimentally laser induced murine choroidal neovessels

Methods 3 groups of 12 adult mice (30g) were submitted to laser impacts breaking slightly the Bruch's membrane and initiating the development of subretinal choroidal neovessels. These laser impacts did not create immediately obvious choroidal lesions leading to blood hemorrhages. One Group of mice was submitted to repeated immunizations up to 5 immunizations by the VEGF Kinoid vaccine associated to a modified Freund's adjuvant solution. One group of mice received only the modified Freund's adjuvant and one group of mice sham injections of PBS in the same conditions.

Results We were able to observe a decrease or even the disappearance of the abnormal choroidal neovessels even in animals that had been immunized but had not reached adequate levels of neutralizing antibodies against VEGF. Transient adverse effects were observed in some mice in all the groups of mice.

Conclusion Obviously The modified Freund's adjuvant solution utilized in this first set of experiments must be avoided or modified in order to avoid adverse effects. The chemistry for the production of the VEGF kinoid production must be improved in order to obtain a better immunizing vaccine.

Commercial interest

■ 4354

Ocular toxoplasmosis recurrences: a single center case report

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Purpose To describe recurrence patterns in a cohort of patients with aqueous humor proven ocular toxoplasmosis, followed during 3 years, at a single referral center.

Methods Retrospective, observational, non comparative case series including 43 patients who suffered from an active episode of toxoplasmic retinochoroiditis during 2005, confirmed by aqueous humour polymerase chain reaction (PCR) positivity and assisted at the Ophthalmology Department of the Pitié-Salpêtrière Hospital in Paris, France. Clinical files were analyzed in terms of signs of intraocular inflammation, number, size and location of retinochoroidal active lesions and scars, presence of ocular complications related to toxoplasmic retinochoroiditis, angiographic and visual field findings and therapeutic management.

Results 20 males and 23 females (mean age 37 year-old) were followed after an episode of toxoplasmic retinochoroiditis confirmed by analysis of ocular fluids. Five of them were immunocompromised and twelve have already experienced at least a previous episode of active toxoplasmic retinochoroiditis. Recurrences occurred in 13 patients (28%) with a mean age of 48 years. These episodes were noted during the first year of follow-up, between 12 and 24 months and between 24 and 36 months in 4, 5 and 3 patients respectively.

Conclusion Recurrences seem to be more frequent if they occur during the first year after the initial episode of retinochoroiditis, especially in older patients. Prospective studies are needed in order to confirm these preliminary data.

■ 4355

Ophthalmic microsporidiosis: the Manchester experience

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Purpose We report cases of ocular and adnexal microsporidiosis diagnosed in Manchester, UK, and review the literature.

Methods The archives of the National Specialist Ophthalmic Pathology (NSOPS) Manchester Laboratory and Health Protection Agency Laboratory (HPA) at Manchester Royal Infirmary were reviewed for cases of microsporidiosis between 1990 and 2009.

Results 8 cases of ocular and adnexal microsporidiosis were identified. Organisms were *Encephalitozoon hellem*, *Encephalitozoon* sp., *Vittaforma corneae*, *Trachipleistophora hominis*, *Nosema* sp. with infection of ocular surface, cornea, nasolacrimal apparatus and nasal sinuses, and eyelid; a historical case of *Microsporidium ceylonensis* keratitis, first reported by Norman Ashton in 1973 was also reviewed. Ages ranged from 11 years (Ashton's case) to 50 years. One case was from an HIV +ve patient, the others were immunocompetent. At least 4 infections were contracted whilst the patient was outside the UK.

Conclusion Microsporidia, minute obligate intracellular parasites related to fungi, infect via a polar tube housed within a highly resistant spore. Microsporidial infection in HIV/AIDS, usually enteric, is the most reported. Antiretroviral treatment has lowered the incidence of enteric microsporidiosis and ocular infection is increasingly prevalent and often not HIV-related. In our cases the majority were immunocompetent individuals. LM can be diagnostic if characteristic refractile ZN-positive spores are seen, but LM does not permit speciation, which usually requires EM. Diligent searching for organisms may be necessary as distribution may be focal. Insufficient data exist for PCR-based diagnosis of most microsporidial species. Sources and mechanisms of microsporidial infection remain speculative.

■ 4357 / 402

TNF α suppresses IFN γ -induced MHC Class II expression in retinal pigmented epithelial cells by down regulating Class II trans-activator mRNA

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Purpose The expression of MHC class II molecules induced by IFN γ on RPE cells plays a crucial role in the development of autoimmune uveitis. As TNF α is commonly coexpressed with IFN γ in this disease, we have investigated the effects of TNF α on the IFN γ mediated MHC II induction in RPE cells.

Methods ARPE-19 were cultured and stimulated with TNF α , IFN γ , IL-1 β and different combination of these cytokines. After cytokine treatment, we have analysed the expression of MHCII and ICAM-1 by flow cytometry. IDO induction was studied by Western blotting. The activation and expression of two proteins involved in IFN pathway: IRF1 and STAT was analysed by Western blot. We have also monitored the effects of TNF α and IFN γ on the expression of CIITA by quantitative RT-PCR.

Results TNF α , but not IL-1, inhibits IFN γ induced MHC class II expression on the surface of ARPE cells. We did not observe an inhibitory effect of TNF α on the expression of ICAM and IDO induced by IFN γ . Similarly, STAT1 phosphorylation and IRF1 induction obtained after IFN γ treatment were not affected by TNF α . On the contrary, we found that TNF α suppresses IFN γ induced CIITA mRNA accumulation.

Conclusion TNF α suppresses IFN γ induced CIITA mRNA accumulation and MHC II expression in human retinal pigment cells.

■ 4356 / 401

Topical levofloxacin 1.5% Is effective in reducing levofloxacin-resistant MRSA and FQ-resistant pseudomonas aeruginosa in keratitis models

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Purpose We compared topical levofloxacin 1.5% (LEV) to standard therapies in reducing levofloxacin-resistant *Staphylococcus aureus* (MRSA) and FQ-resistant *Pseudomonas aeruginosa* (PA) in keratitis models.

Methods Both corneas of 32 NZW rabbits were intrastromally injected with 1000 CFU of MRSA (LV MIC=32 μ g/ml; van MIC=2 μ g/ml). After 4 hours (onset), 8 rabbits were sacrificed and the corneas were homogenized for colony counts. 24 rabbits were divided into 3 topical treatment groups: 1) LEV, 2) vancomycin 5%, and 3) saline. Treatment consisted of drops every 15 minutes/5 hours (21 drops). One hour after treatment, the rabbits were sacrificed and the corneas were homogenized for colony counts. Similar to MRSA, 32 NZW rabbits were intrastromally injected with 1000 CFU of PA (LV MIC=32 μ g/ml, tob=0.75 μ g/ml). After 16 hours (onset), 8 rabbits were sacrificed and the corneas were homogenized for colony counts. 24 rabbits were divided into 3 topical treatment groups: 1) LEV, 2) tobramycin 1.4%, and 3) saline. Treatment consisted of drops every 15 minutes/1 hour and every 30 minutes/7 hours (19 drops). One hour after treatment, the rabbits were sacrificed and the corneas were homogenized for colony counts. The CFU data were analyzed non-parametrically.

Results LEV significantly reduced more MRSA than vancomycin 5%, and both significantly reduced more MRSA than the onset and saline control (p<0.05). LEV was as effective as tobramycin 1.4% in reducing PA, and both significantly reduced more PA than the onset and saline control (p<0.05).

Conclusion LEV was more effective for reducing MRSA and as effective for reducing PA as standard therapies in rabbit keratitis models.

Commercial interest

■ 4358 / 403

In vitro antimicrobial effect of vitreous endotamponading substances

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Purpose To demonstrate the in vitro bactericidal, bacteriostatic or inert role of endotamponading substances.

Methods Clinical isolates of *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Clostridium* sp were cultured on blood agar plates in sulphur hexafluoride (SF6) and perfluoropropane (C3F8) atmospheres and under silicone oil (SO) and liquid perfluorocarbon (LPFC). The same germs were cultured under aerobic and anaerobic atmospheres as controls.

Results SO, SF6 and C3F8 did not significantly inhibit the growth of anaerobic bacteria but markedly reduced the growth of aerobic germs, as compared with aerobic conditions. The growth of the bacteria under SO and SF6 and C3F8 was very similar to that observed under anaerobic conditions. LPFC did not affect the growth of aerobic or anaerobic bacteria.

Conclusion Endotamponading gases and liquids seem to improve the visual and anatomical outcome of infected eyes and may limit the proliferation of aerobic germs in vitro. It is not known to what extent the antimicrobial effect of these substances would appear in vivo since oxygen diffusion from the blood could allow the growth of aerobic germs.

■ 4359 / 404

An epidemiologic analysis of staphylococcus aureus-associated keratitis in Boston

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Purpose *S. aureus* is a normal commensal of the human skin and nasopharynx. It is therefore of interest to determine whether *S. aureus* keratitis is caused by a subset of these organisms. In this study, the phenotypic and genotypic characteristics of *S. aureus* keratitis isolates were analyzed.

Methods All *S. aureus* clinical isolates were prospectively collected over a 24 month period at the MEEI (2006-2008). The diagnosis of clinical keratitis and associated risk factors was by medical record review. Keratitis-associated *S. aureus* strains were assessed for:

1. antibiotic susceptibility,
2. biofilm robustness by gentian violet staining using an in vitro microtiter plate assay, and
3. genetic lineage by multi-locus sequence typing (MLST).

Results 26 cases of keratitis were identified from the 600 *S. aureus* clinical isolates. Risk factors associated with *S. aureus* keratitis included trauma, prior surgery, soft contact lens wear, and the presence of a foreign body. Ocular surface disease does not appear to be an independent risk factor. All 26 isolates were tetracycline- and trimethoprim-sulfamethoxazole- sensitive. All the MRSA strains were found to be ciprofloxacin-resistant (10/26). Nearly one-half of all the *S. aureus* keratitis-associated isolates were caused by a single clone, ST5. Both methicillin sensitive and resistant *S. aureus* strains were represented within ST5.

Conclusion These results suggest that there may be specific *S. aureus* lineages which possess phenotypic and genotypic characteristics that enable *S. aureus* to more effectively cause sight-threatening keratitis. Future work will examine their virulence traits and a comparison to commensal *S. aureus* strains.

■ 435a / 405

Comparative assessment of *S. aureus* microbial biofilm inhibition by an N-alkyl-polyethyleneimine covalently attached to PMMA or titanium in the Boston Keratoprosthesis

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Purpose Biofilms are matrix-associated microbial communities adherent to either biological surfaces or abiotic surfaces. They account for the majority of device-associated infections. Our goal herein is to minimize bacterial adherence and biofilm formation by comparative analysis of new polycations bound to bio-prosthetic ocular-associated materials, poly (methyl methacrylate) (PMMA) or titanium, using the Boston KPro as a model system.

Methods Using *S. aureus* ocular-associated clinical isolates, a quantitative assessment of microbial biofilm formation by linear N,N-dodecyl,methyl-polyethyleneimine (DMPEI) (217 kDa) covalently bound to PMMA or titanium compared to the parent PMMA or Ti, respectively, has been performed using confocal laser scanning and electron microscopies. In addition, DMPEI-bound materials have been screened for corneal toxicity in both cell tissue culture and rodent models, and as compared to the original materials.

Results A marked inhibitory effect in *S. aureus* biofilm formation on DMPEI-derivatized materials compared to the parent PMMA (3-4 fold) and Ti (2-fold), without conferring additional epithelial cell cytotoxicity in vitro, has been observed. Furthermore, we have found no additional tissue reactivity, and possibly even a protective effect, with DMPEI-derivatized materials in vivo.

Conclusion We found that covalent derivatization with DMPEI of PMMA and Ti greatly reduces *S. aureus* biofilm formation in vitro compared to the parent materials. There was no additional cytotoxicity seen both in vitro and in vivo. Future studies will evaluate DMPEI-derivatized materials for in vivo antimicrobial efficacy.

■ 4361

Pigmented eyelid lesion - unexpected findings

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Purpose To present the clinical and histologic findings of an unusual eyelid lesion.

Methods A 63-year-old patient was referred to our Department with a small painless nodular tumour of his left lower eyelid. The lesion had been present for about six months and had increased in size during the last weeks. Slit lamp microscopy revealed a lesion with a smooth surface near the lid margin. On eversion of the eyelid there was some associated pigmentation on the tarsal conjunctiva which had caused the referring Ophthalmologist to suspect a melanocytic process. The lesion was removed by wedge resection and examined by light microscopy and immunohistochemistry.

Results Findings will be demonstrated at the meeting.

Conclusion The lesion presented here is a rare entity but should be considered in the differential of some fairly common eyelid tumours.

■ 4362

Uveal melanoma in a 12-year old child

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ABSTRACT NOT PROVIDED

■ 4363

Irido-cilio-choroidal melanoma in a 5-year old child

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ABSTRACT NOT PROVIDED

■ 4364

A child with a melanocytic ciliary body tumour

KIVELÄ T
Helsinki

ABSTRACT NOT PROVIDED

■ **4365**

Local resection of end-stage choroidal melanoma

*DAMATO B
Liverpool*

ABSTRACT NOT PROVIDED

■ **4366**

**Large retinal hemangioma treated in second hand by
betabrachytherapy, on a single eye of a young adult**

*GRANGE JD, KODJIKIAN L
Lyon*

ABSTRACT NOT PROVIDED

■ **4367**

Sudden loss of vision 15 years after renal transplantation

*COUPLAND SE, THUMANN G
Liverpool*

ABSTRACT NOT PROVIDED

■ **4368**

Orbital mantle cell lymphoma

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ABSTRACT NOT PROVIDED

■ 4411

Complex regulation of choroidal blood flow during combined changes in blood pressure and IOP

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Purpose In the recent years it has been shown that the human choroid shows some regulatory capacity during an artificial increase in intraocular pressure (IOP) as well as during an exercise induced increase in mean arterial pressure (MAP). The purpose of the present study was to test the hypothesis that human choroidal blood flow (ChBF) may depend, not only on ocular perfusion pressure (OPP), but also on absolute levels of MAP and IOP.

Methods In a clinical study in 28 healthy subjects OPP was varied by elevating IOP during a squatting-induced increase in MAP. IOP was raised in stepwise increments by using the suction cup method. Subfoveal choroidal blood flow (VhBF; laser Doppler flowmetry), MAP, and IOP were assessed, and OPP was calculated as $(2/3)(MAP - IOP)$. For correlation analysis, data from all subjects were pooled according to IOP and MAP values, and correlation analyses were performed.

Results When data were grouped according to IOP, no correlation was observed between ChBF and MAP; but ChBFs were lower, the higher the IOP ($P < 0.001$). When data were grouped according to MAP, a significant correlation was found between ChBF and IOP ($P < 0.001$), but correlations were independent of MAP.

Conclusion These data confirm previously published observations that the choroid shows some regulatory capacity during changes in OPP. In addition, the data indicate that the choroid regulates its blood flow better during exercise-induced changes in MAP than during an experimental increase in IOP.

■ 4413

Age-related macular degeneration: hemodynamic changes

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Purpose Metabolic changes of the RPE associated to the dysfunction of choriocapillaries(CC)/RPE complex may induces the AMD-related changes. Additional vascular changes in the choroid potentially have deleterious effects on the RPE.

Methods Quantification of CC number and lumen diameters in cross sections and alkaline phosphatase (APase) flat-embedding technique, expressing high constitutive APase activity in choriocapillaris and choroidal veins on human RPE/Bruch's Membrane/CC complex, significantly contributed to the analysis of the choroidal vasculature. Laser Doppler flowmetry (LDF) data provided additional information on the assessment of hemodynamic changes in AMD.

Results Choroidal vascular density reduction and significant vasoconstriction of the choriocapillaries, occurs during the evolution of AMD. In eyes with geographic atrophy, the RPE degenerates first while CC loss is secondary to RPE degeneration. In eyes with exudative AMD, degeneration of the CC layer occurs while RPE is still functional. LDF data indicated choroidal blood flow decrease according to age and the degree of severity of AMD; the decrease in flow preceding the formation of choroidal CNV, strongly suggest that these changes may have a role in the development of CNV. As a result of vascular dysfunction, the choroidal blood flow is dysregulated in patients with neovascular AMD. The choroidal watershed zone (WZ) courses through the fovea more often in patients suffering from AMD than in age-matched controls, particularly in the presence of CNV. Choroidal neovascularisation usually arises within these WZ.

Conclusion The role of choroidal ischemia in the pathophysiology of AMD is supported by the observed choroidal microcirculation anatomical and functional abnormalities.

■ 4412

Evaluation of choroidal blood flow after treatment of retinal diseases

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Purpose this review aims to summarize studies which assessed the effect of treatment on choroidal blood flow.

Methods this presentation will focus on studies using the laser Doppler flowmeter for the analysis of choroidal blood flow parameters (velocity, volume and flow) before and after treatment. Therapies have been assessed in different ocular disease, such as age-related macular degeneration (laser photocoagulation therapy, photodynamic therapy, transpupillary thermotherapy; sildenafil citrate, niacin, pentoxifylline), diabetes mellitus (panretinal photocoagulation, intravenous C-peptide infusion), retinal vein occlusions (isovolemic hemodilution), macular edema (diclofenac), inflammation (corticosteroid), retinal detachment (surgery) or glaucoma (nimodipine, endothelin receptor antagonist, bimatoprost, timolol, trabeculectomy).

Results this paper will give insight to the effects of laser treatment (laser photocoagulation, photodynamic therapy), surgery (scleral buckling, trabeculectomy, ocular anesthesia) or systemic drugs on the choroidal blood flow. Methodological considerations will be analyzed, such as the calculation of the sensitivity of the experiments, the comparisons of different groups with or without randomization.

Conclusion laser Doppler flowmetry is a useful and a non invasive technique to study the effect of treatment on choroidal blood flow. In ocular disease, investigators should be aware of the tissue scattering changes associated with a retinal or choroidal disease and the necessity of a controlled foveal fixation.

■ 4414

Subfoveal choroidal blood flow and central retinal function in retinitis pigmentosa

FALSINI B

Rome

ABSTRACT NOT PROVIDED

■ 4415

Autoregulation in the choroid

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Purpose To compare subfoveal choroidal blood flow (ChBF) in sitting and supine position in normal volunteers.

Methods ChBF was measured with laser Doppler flowmetry in 22 healthy volunteers (mean age \pm SD: 24 \pm 5 years). Six independent measurements of choroidal blood flow were obtained in one randomly selected eye of each subject. Subsequently, the subjects assumed a supine position for 30 minutes and a new series of 6 measurements was obtained. Parallel hereto, systemic blood pressure and intraocular pressure were measured. Ocular perfusion pressure (OPP) was calculated based on formulas derived from ophthalmodynamometric studies. The influence of changing OPP on the change in ChBF was assessed in a linear regression analysis.

Results The coefficient of variation for ChBF was 10.28% and 9.58% in the sitting and the supine position respectively. ChBF decreased by 6.6% ($p=0.0017$) in the supine position. The estimate for ophthalmic blood pressure in the supine position was adjusted to obtain a result of no change in OPP for no change in ChBF, yielding an average decrease for the estimate of OPP of 6.7% ($p=0.0002$). Change in OPP correlated significantly with change in ChBF ($R^2: 0.20$; $p=0.036$) with a slope for the regression line of 1.04.

Conclusion The comparable degree of change in ChBF and OPP and the linear relationship between the two parameters suggest a passive response of the choroidal circulation to the posture change. In contrast, the OPP estimates suggest a marked buffering of the change in perfusion pressure by the carotid system, compatible with a close control of the gradient in perfusion pressure between the heart and its branches within the carotid system.

■ 4416

Choroidal blood flow and retinal ganglion cell function in early glaucoma

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Purpose To assess subfoveal choroidal blood flow in patients with early manifest glaucoma (EMG) and to compare blood flow with functional measures of retinal ganglion cell (RGC) integrity.

Methods Subfoveal choroidal blood flow was determined by confocal, real-time laser Doppler flowmetry in 25 EMG patients (< -6 dB Humphrey mean deviation, age range: 42-64 years, visual acuity: 0.8-1.0) and in 20 age-matched controls. All patients had a therapeutically (topical beta-blockers with or without a prostaglandin) controlled intraocular pressure (IOP < 20 mmHg). Subfoveal choroidal blood volume (ChBVol), velocity (ChBVel) and flow (ChBF) were determined as the average of three 60 sec recordings with changes in the DC $< 10\%$ between the recordings (DC measures the intensity of the light scattered by the tissue and red blood cells in the illuminated volume). In all patients and controls pattern electroretinograms (PERGs) were recorded according to a standardized protocol.

Results In EMG patients, average ChBVel and ChBF were reduced by 31 and 35%, respectively ($p < 0.01$) compared to control values. No significant difference in ChBVol was found between the two groups. PERG amplitudes were reduced by 40% ($p < 0.01$) in EMG patients compared to controls. No correlation was found between any of the choroidal flow parameters and PERG data or IOP values.

Conclusion The results suggest a significant alteration of subfoveal choroidal hemodynamics in EMG patients, involving both ChBVel and ChBF. These changes do not appear to be associated with the severity of functional retinal ganglion cell loss. Our findings may have implications for the pathophysiology of early glaucomatous damage and its treatment.

■ 4421

Biosynthetic corneas – evaluation in humans

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Collagen-based biosynthetic corneas, designed to mimic the extracellular matrix of the corneal stroma have been developed and extensively evaluated in animal models over the last 7 years. Human recombinant collagen type III (RHC III) was crosslinked with water-soluble carbodiimides and fabricated into optically transparent corneal substitutes for transplantation. Following study approval of the Medical Product Agency, Sweden and the Human Ethics Committee, University of Linköping, Sweden, a Phase I study was initiated. 10 patients who were scheduled for corneal grafting were enrolled into the study. Nine had keratoconus and one had a deep scar following Pseudomonas keratitis. A central 6 mm diameter deep lamellar button was excised and was replaced by a 6.25 mm diameter 500 µm thick construct. Six overlying sutures were used to anchor the graft. Topical 0.1% dexametasone and chloramphenicol was used for the first 1 month postoperatively. The sutures were removed after 5-7 weeks. The patients were followed clinically and evaluated for UCVA, BSCVA and VA with contact lenses. Corneal touch sensitivity (Cochet-Bonnet) and tear production (Schirmer) were tested. Photography, OCT (Visante), topography (Orbscan II) and in vivo confocal microscopy (Heidelberg) was documented. After 3 months all patients had stably epithelialized and implants were anchored by recipient keratocyte ingrowth. The mean BSCVA at 6 months (20/133) improved slightly at 12 months (20/90). The mean BCLCVA was 20/50 at 12 months and was notably better in younger patients (mean of 20/40 in the 5 youngest). One patient had BCLVA of 20/20 at 12 months. The mean central corneal thickness was stable between 3 and 12 months at about 400µm. The mean 5min Schirmer values were 20 ± 10mm in operated eyes and 17 ± 8 mm in fellow eyes. At 12 months the mean touch sensitivity was 25mm in operated eyes and 60mm in fellow eyes, which was the same as in penetrating grafts. In-vivo confocal microscopy revealed the ingrowth of corneal nerves at the subbasal epithelium. We have shown for the first time that bioengineered collagen-based corneal substitutes are fully compatible and promote regeneration of corneal cells. The 18 months follow-up results will be presented as well.

■ 4423

Glaucoma associated with KPros

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Purpose Glaucoma is one of the most serious problems in Keratoprostheses. Already glaucoma is very frequent in patients with severe changes of the ocular surface requiring keratoprosthesis surgery. About 50% of those patients have pre-existing secondary glaucoma. Preoperatively all efforts should be done to detect its presence very early on. In KPro detection of glaucoma with traditional aids is difficult. A rough estimation of the tension by digital palpation will be the only available method. All indirect clues such as the medical history of glaucoma, echographic signs of disc cupping and anterior synechiae and examination of the visual field should be considered.

Methods The incidence of postoperative secondary glaucoma vary among the different kinds of prostheses according to the surgery affecting the anterior segment and the long term anatomic results. In Osteo-Odonto-Keratoprostheses (OOKP) the most vision threatening complication is a primary or secondary glaucoma, due to the extended interventions required in the anterior segment. In Boston Keratoprostheses the risk seems to be somehow less.

Results As the absorption of topical anti-glaucomatous medication will not reach the inner eye because of the anatomic barrier in KPro the only promising possibilities of treatment are systemic carbonic anhydrase inhibitors, different kinds of aqueous shunts and endo-cyclo-laserphotocoagulation.

Conclusion Glaucoma is still a mayor problem in KPro. PS: This lesson will be presented at the KPro-Meeting!!

■ 4422

Keratoprosthesis surgery: Eastern European and Russian devices

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 (4) S.Fyodorov Eye Microsurgery Complex State Institution, Moscow

Purpose To present the development and current status of keratoprosthesis surgery in Eastern Europe.

Methods Collection of data from coauthors and other surgeons involved in k-pro surgery.

Results Large numbers of surgeries were performed in Filatov's Institute in Odessa (Ukraine), where over 1000 different types of devices developed by Puchkovskaya, Yakimienko and Golubenko were implanted since 1966. The last model, s.c. "universal separable device" was implanted in over 750 with the best results (extrusion occurred in about 2.5% cases). K-pro devices in Russia were mostly developed by S. Fyodorov. Moroz, V. Zuyev, M. Krasnov, V. Volkov, R. Gundorova, N. Ushakov and V. Bedilo. Over 1500 surgeries since 1969 resulted in the visual acuity improvement in 94% of cases. Haptics were made of titanium, stainless steel and also biocompatible materials (xenopericardium). In Poland about 100 surgeries were performed using mostly Russian and Ukrainian devices. The results were less favorable than in countries of origin. Small numbers were also implanted in other East European states.

Conclusion In former Soviet Union keratoprosthesis surgery was well developed in selected centers (Moscow, Odessa). Surgeons in these places have gained extraordinary experience performing hundreds of surgeries. The results presented by the authors were excellent, however they were less favorable in the hands of surgeons from other countries.

■ 4424

Improving the optics of KPros

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 (2) Brighton

Purpose The purpose of this talk is to review the clinically relevant optical and visual requirements of keratoprostheses.

Methods Modelling of designs using commercial and purpose-written optical design software as well as clinical measurements on a small number of patients implanted with the osteo-odonto keratoprosthesis optical cylinder.

Results The important visual optical properties of any keratoprosthesis optic are the foveal image quality (affecting the acuity) and the field of view. These fundamentally depend upon the pupil size, and the length and diameter of the optic since the power must be fixed to give emmetropia. The axial image quality can be changed by controlling the spherical aberration. However, maximising axial (foveal) image quality causes poorer off-axis image quality potentially reducing the effective visual field. Shorter, larger diameter optics help improve the visual field as well as consideration of off-axis image quality.

Conclusion The theoretical properties of keratoprosthesis optics must be balanced against other clinical requirements such as longterm complications and stability. The clinical requirements provide the constraints on the optical design. However, discussions can usefully happen between surgeons and those involved in the design of the optics to give the best possible visual rehabilitation.

■ 4425

Glaucoma treatment in MOOKP

TALONI M, FALCINELLI G

Glaucoma is the most common complication following modified osteo-odonto-keratoprosthesis (MOOKP). Between 1973 and 2007, 266 eyes have been operated by the same surgeon (G.Falcinelli) with a modified osteo-odonto-keratoprosthesis.

Before implantation, 94 patients had a preexisting glaucoma (36%).

Over 266 MOOKPs, 69 patients (26%) have to be treated for glaucoma (53 relapsed, 16 ex novo glaucoma). 28 patients were stabilized, 16 were slightly worse, 23 worsened, 3 had an absolute glaucoma.

Surgical approach applied was retroequatorial drainage for 12, double thread cyclodiastasis for 11, 3 transcleral photocoagulation, 1 diathermy of the ciliary body, 3 endo-cyclo photocoagulation, 1 Ahmed valve. The results will be discussed. But certainly an inaccurate surgical procedure can facilitate the onset of glaucoma after MOOKP.

■ 4427

Does imaging help with preventing extrusion?

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Both clinical and radiological methods can be used for early detection of resorption in OOKP patients; this is influential in preventing serious complications such as extrusion and endophthalmitis. Radiologically, use of either multidetector computed tomography (MDCT) or electron beam tomography (EBT) is valuable in identifying laminar resorption. A novel approach was recently adopted in Sussex Eye Hospital, using volume rendering software in processing previously obtained MDCT images, to calculate volume of the OOKP lamina rather than 2D measurements. We present the results of an observational retrospective case series study to illustrate the use of this approach. We describe how it can be used to calculate percentage change in volume of the lamina over time and how this can be correlated with clinical laminar resorption. We will also discuss further recommendations to build upon this advance.

■ 4426

An antibiotic releasing contact lens

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Purpose To characterize a drug-eluting contact lens designed to release ciprofloxacin to the eye in a controlled manner for an extended period of time.

Methods Thin films, containing ciprofloxacin or fluorescein encapsulated in PLGA (Poly[lactic-co-glycolic acid]), were coating by pHEMA (poly[hydroxyethyl methacrylate]) by ultraviolet light polymerization, creating prototype contact lenses. The films were characterized by scanning electron microscopy. Release studies were conducted in phosphate buffered saline at 37°C with continuous shaking. Ciprofloxacin eluted from the contact lens was studied in an antimicrobial assay to verify antimicrobial effectiveness.

Results Ciprofloxacin and fluorescein were released from the contact lenses in a controlled manner, demonstrating zero-order release kinetics under infinite sink conditions for over 4 weeks. The rate of drug release was controlled by modifying either the ratio of drug to PLGA or the molecular weight of the PLGA employed. Both the PLGA and the pHEMA affected release kinetics. Ciprofloxacin released from the contact lenses inhibited ciprofloxacin-sensitive *Staphylococcus aureus* at all time-points tested.

Conclusion A thin drug-PLGA film coated with pHEMA could potentially be used to create contact lenses which can serve as a platform for ocular delivery of antibiotics and other medications, with widespread therapeutic applications.

■ 4428

The clinical psychologist's role in the OOKP clinic. A one year review

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(2) *Brighton*

OOKP Surgery places complex Physical and Psychological demands on patients. Attention to Psychological factors is likely to facilitate good outcome and improve Quality of Life. This presentation outlines an innovative addition to the OOKP service at Sussex Eye Hospital, Brighton UK, incorporating a Clinical Psychologist as a member of the OOKP medical team. It will describe the first year's work including a study which has identified four subgroups of patients presenting for OOKP and the psychological needs of each group. The presentation describes how the service aims to address the particular psychological needs of these patient groups from assessment to psychological follow up. The presentation will also include a summary of a second Qualitative Interview study looking at patients' reports of their experiences of undergoing OOKP from a bio-psychosocial perspective. The National Health Service, UK seeks to use patient experience and feedback to shape clinical services and the implications of the outcomes of the research for the service will be discussed.

■ 4451

Introduction

HERBERT CP

ABSTRACT NOT PROVIDED

■ 4453

Uveitis caused by fastidious bacteria

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Purpose The etiologic evaluation of uveitis is frequently unsuccessful if only noninvasive methods are used. We conducted a prospective study to evaluate systematic screening for pathogens of uveitis.

Methods All patients with uveitis referred to the participating tertiary ophthalmology departments from January 2001 to September 2007 underwent intraocular and serum specimen collection. The standardized protocol for laboratory investigations included universal polymerase chain reaction (PCR)-based detection of any bacteria and mycoses, specific PCR-based detection of fastidious (difficult-to-grow) bacteria and herpes viruses, and culture of vitreous fluid. Sera were tested for fastidious bacteria.

Results Among the 1321 included patients, infection was diagnosed in 147 (11.1%) patients: 78 (53%) were caused by fastidious bacteria that included spirochetes, Bartonella species, intracellular bacteria (Chlamydia species, Rickettsia species, Coxiella burnetii), and Tropheryma whipplei; 18 by herpes viruses; and 9 by fungi. Bartonella quintana, Coxiella burnetii, Paracoccus yeei, Aspergillus oryzae, and Cryptococcus albidus were found to be associated with uveitis for the first time, to our knowledge.

Conclusion We recommend applying a 1-step diagnostic procedure that incorporates intraocular, specific microbial PCR with serum analyses in tertiary centers to determine the etiology of uveitis.

■ 4452

Emerging and poorly known viral inflammatory eye diseases

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Results Arthropod vector borne diseases are among the most important emergent infections. They include a wide variety of bacterial, viral, and parasitic diseases that are transmitted to humans by the bite of mosquito, tick, or other arthropod. Most of them are prevalent in tropical and subtropical areas, but they tend to spread into new regions mainly due to increasing temperatures worldwide, movement of people, increasing human population densities, wider dispersal of competent vectors, and transportation of goods and animals. Numerous arthropod vector borne diseases have been associated with uveitis. Among them, specific viral diseases recently emerged as important causes of uveitis in the developing and developed world. They include West Nile virus (WNV) infection, Rift Valley fever (RVF), dengue fever (DF), and Chikungunya. These viral diseases have been recently associated with an array of ocular manifestations, including anterior uveitis, retinitis, chorioretinitis, retinal vaculitis, and optic nerve involvement. Proper clinical diagnosis of any of these infectious diseases is based on epidemiological data, history, systemic symptoms and signs, and the pattern of uveitis. The diagnosis is usually confirmed by detection of specific antibody in serum. A systematic ocular examination, showing fairly typical findings, can help establish an early clinical diagnosis of a specific systemic viral infection while serologic testing is pending. Prevention remains the mainstay for control of arthropod vector borne viral diseases.

■ 4454

Serpiginous choroiditis revisited

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Purpose Serpiginous Choroiditis (SC) is a subtype of posterior uveitis with often unfavorable visual prognosis, often requiring intensive immunosuppressive treatment. Histological and clinical evidence has been reported linking tuberculosis to cases of chorioiditis and specifically to serpiginous-like chorioiditis. A potential tuberculous origin is of concern as the treatment would be completely different. Tuberculin skin test has limited power for diagnosing tuberculosis since active disease cannot be differentiated from immunity and because of cross-reactivity with bacillus Calmette-Guérin (BCG). It is one of the oldest diagnostic tests still in use worldwide with variable sensitivity and specificity depending on the cut-off used and the population screened. Therefore, we used an interferon- γ release assay (QuantiFERON TB-Gold; Cellestis, Australia) to obtain a diagnosis of tuberculosis in patients with serpiginous like chorioiditis.

Methods Quantiferon is an approved, antigen-specific test that utilizes synthetic peptides representing M. tuberculosis proteins. After incubation, interferon- γ secreted by T-lymphocytes in response to these antigens is measured.

Results In our cohort 52 % of SC patients were tested positive. Patients with SC also showed higher absolute Tb antigen responses as compared to Quantiferon positive patients with other uveitis subtypes.

Conclusion Our findings support a tuberculous origin in SC. Other groups also found evidence of Tb association with SC, proven by radiologic imaging. These patients seem to improve with anti-Tb treatment. The diagnostic significance of Quantiferon and other Tb testing in patients with SC and treatment implications will be discussed.

Posters

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- Posters 301 - 370, exhibited on Friday 187
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■ 201

Magnetic resonance Imaging vs. ultrasound biometry in the equatorial plane in human eyes

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Purpose Our purpose was to assess the reliability of ultrasound (US) measurements versus magnetic resonance imaging (MRI) in the equatorial plane in human eyes.

Methods Four patients who later underwent retinal prosthesis implantation and 8 healthy volunteers aged between 20 and 50 years of age (mean (SD) age 36.8 (8.0) years) were included in the study. One operator measured the axial length and the vertical equatorial diameter (in maximal down gaze) with US applying a contact 10 MHz A-scan transducer. MR measurements (Siemens MAGNETOM Trio, A Tim System) were taken using five different sequences. MRI and US data were compared with paired sample t-test and Pearson correlation analysis. The intraclass correlation coefficient (ICC) was calculated.

Results Axial diameter (mean \pm SD) was measured by US (24.45 \pm 1.00 mm), and by MRI (24.22 \pm 1.08). Vertical diameter was also measured by US (24.12 \pm 1.10) and by MRI (24.22 \pm 1.01 respectively). No significant difference was found between US and MRI data in axial and vertical planes ($p=0.134$, 0.598 respectively). Significant correlation could be found both in axial ($p=0.01$, $r=0.933$) and in the vertical planes ($p=0.02$, 0.831). Intraclass correlation coefficient (ICC) revealed good reliability with high consistency (axial: 0.963 , vertical: 0.906) and absolute agreement values (axial: 0.955 , vertical: 0.912).

Conclusion Our results demonstrated that both the US and the MRI can be used to assess the equatorial diameter of the human eyeball. US measurements revealed high correlation with MRI data with respect to the equatorial plane and axial length.

■ 203

Comparison of the corneal thickness measured by anterior segment optical coherence tomography and specular microscope versus ultrasound pachymetry

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Purpose To compare measurements of central corneal thickness (CCT) between the noncontact and contact ultrasound pachymetry and to define endothelial cells density in normal eyes.

Methods 60 eyes of 30 clinically healthy persons – 20 women and 10 men, whose age ranged from 22 to 26 years, were studied. Measurements were performed by specular microscope, anterior segment optical coherence tomography and ultrasound pachymetry. Results obtained with these devices were compared. For statistical analysis of Pearson correlation coefficient and Bland-Altman plot was used MedCalc software, version 8.1.

Results The mean central corneal thickness (CCT) as measured by CP was $544.9 \pm 43.2 \mu\text{m}$ of the right eye and $544.7 \pm 41.1 \mu\text{m}$ of the left eye. The mean CCT as measured by OCT was $544.2 \pm 42.1 \mu\text{m}$ of the right eye and $549.50 \pm 44.5 \mu\text{m}$ of the left eye. The mean CCT as measured by SM was $558.87 \pm 45.8 \mu\text{m}$ of the right eye and $556.93 \pm 45.6 \mu\text{m}$ of the left eye. Pearson correlation coefficient was statistically significant ($0.919 - 0.964$) for all compared groups. By Bland-Altman plot method statistically significant deviation was not found for any set of groups. The mean endothelial cell density was $2917.3 \pm 532.1 \text{ cell}/\text{mm}^2$ for the right and $2947.6 \pm 331.5 \text{ cell}/\text{mm}^2$ for the left eye respectively.

Conclusion

1. The results of central corneal thickness measurements obtained using all three methods are in linear dependence.
2. The intrasession variation of central corneal thickness measured by specular microscope was higher than that of contact method and Visante OCT.

■ 202

The correlation between central corneal thickness, ocular and general parameters

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Purpose To evaluate central corneal thickness (CCT) of normal Lithuanian population, to describe the relationship between CCT, height, weight, body mass index (BMI), intraocular pressure (IOP), refraction ability.

Methods In a pilot study 259 adults- 125 men and 134 women, whose age ranged in 18-68 years, were studied. Height, weight and body mass index was determined using a standardized protocol. Non-cycloplegic refraction was determined by an autorefractor (KR-8100P; TOPCON). The intraocular pressure was measured by applanation tonometer (Reichert Tono-pen XL). CCT was measured by contact ultrasound pachymeter (Pocket Pachymeter, Quantel Medical, CP), respectively. Statistical analysis was made using SPSS 17.0 software. Correlations between groups were determined using Pearson two sided correlation coefficient.

Results The mean CCT of males was $549.0 (\pm 33.6) \mu\text{m}$ for the right eye (OD) and $549.9 (\pm 33.8) \mu\text{m}$ for the left eye (OS). The mean CCT of females was $542.6 (\pm 34.9) \mu\text{m}$ for OD and $543.1 (\pm 35.2) \mu\text{m}$ for OS. Mean height of the examined persons was $173.06 (\pm 8.27) \text{ cm}$; mean weight – $68.69 (\pm 11.84) \text{ kg}$; mean value of the body mass index – $22.83 (\pm 2.95)$; mean intraocular pressure – $16.07 (\pm 2.74) \text{ mmHg}$; mean refraction ability – $-1.56 (\pm 2.21) \text{ D}$. Pearson two sided correlation coefficient between CCT and subjects' age was 0.00 ; CCT and height – 0.108 ; CCT and weight – 0.093 ; CCT and BMI – 0.05 ; CCT and IOP – 0.26 ; CCT and refraction – 0.047 .

Conclusion 1. No significant difference in mean CCT was found between the right and left eyes or between male and female subjects. 2. CCT correlated with height, weight and IOP; but not BMI or refraction ability in the overall study group. 3. CCT significantly correlated with height.

■ 204

Ultrastructural features of tree shrew cornea-collagen fibrils and proteoglycans

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Purpose The unique organisation of collagen fibrils and proteoglycans maintain the transparency of normal cornea. We report here ultrastructural features of collagen fibrils and proteoglycans of tree shrew cornea.

Methods Tree shrew corneas of 5, 6 and 10 week old animals were fixed in 2.5% glutaraldehyde containing cuproinic blue in sodium acetate buffer. The tissue was processed for electron microscopy. Soft imaging programme 'analySIS LS Professional' was used to analyse collagen fibril diameter and proteoglycans area.

Results Tree shrew corneas consist of 5 layers, the epithelium, Bowman's layer, stroma, Descemet's membrane. The epithelium was composed of squamous cells, wing cells and basal cells. The Bowman's layer was $5.5 \mu\text{m}$ thick and very similar to normal human Bowman's layer. The stroma was $258 \mu\text{m}$ thick and consisted of collagen fibril lamellae. Lamellae interlaced each other in the anterior stroma, but ran parallel to each other in the middle and posterior stroma. Longitudinally running collagen fibrils showed banding and were decorated with proteoglycan filaments of area size 390 nm^2 . The collagen fibril diameter was 39 nm . Very small electron dense particles were observed within the collagen fibrils. Descemet's membrane has only banded region. Endothelial cells contained all the normal cell organelles.

Conclusion The structure of the tree shrew cornea is very similar to normal human cornea. The collagen fibril of the former uniquely showed the presence of electron dense particles within collagen fibrils. These particles could be proteoglycans stained with cuproinic blue. Similarities of tree shrew cornea with human cornea suggest that it can be a good model to study human corneal diseases.

■ 205

Treatment of ocular surface injuries by the transfer of limbal and mesenchymal stem cells growing on nanofibrous scaffolds



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Purpose To use nanofibrous scaffolds to grow and transfer limbal stem cells (LSC) and mesenchymal stem cells (MSC) to treat ocular surface injuries in a mouse experimental model.

Methods Nanofibrous scaffolds were prepared by electrospinning procedure from a polyamid 6/12 (PA6/12). This polymer was selected on the basis of stability of nanofibres in aqueous solutions, biocompatibility and the best properties as a matrix for the growth of LSC and MSC. LSC and MSC labeled with a vital fluorescent dye PKH26 and grown on nanofibrous scaffold were transferred on the damaged eye surface, where their seeding and surviving were monitored. The effects of transferred cells on local inflammatory reactions were assessed by Real-time PCR.

Results The morphology, growth properties and viability of LSC and MSC on PA6/12 nanofibres were comparable with those on plastic. Transfer of LSC and MSC growing on nanofibrous scaffolds on the damaged ocular surface significantly inhibited local inflammatory reactions and supported the healing process.

Conclusion The nanofibers prepared from polymer PA6/12 represent a convenient scaffold for LSC and MSC growth and transfer to treat SC deficiencies and various eye surface injuries.

■ 207

The influence of pollen proteases on human conjunctival cells

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Purpose During pollen-seasons allergy-like symptoms can also be observed in proven non allergy sufferers. Pollen proteases are thought to be responsible for consecutive conjunctival irritation. We investigated the influence of a well known aggressive pollen species, birch pollen (*Betula pendula*) on human conjunctival cell cultures. This study is an approach to SNAC (seasonal non allergic conjunctivitis) syndrome.

Methods Human cultivated conjunctival cells, so called CHANG-cells, were incubated with extracts of birch pollen. Zymography was carried out in order to investigate the proteolytic activity of the pollen. Cytomorphological changes were analysed using the CASY1 Cell Counter. Via MTS-assay the cell viability was quantified. The viability of the cells, which were incubated with pollen extract, was compared to the viability of the control cells and the results were statistically evaluated.

Results Depending on the pollen extract concentration and the incubation period, the treatment of CHANG-cells with pollen extract of birch induced a highly significant decrease of the cell viability.

Conclusion Cell damage by pollen proteases is an approach to explain conjunctival irritation in proven non-allergic persons during the pollen season. A reason why not all persons are affected by the SNAC syndrome at the same extent, could be differences in the concentrations of antiproteases present on the ocular surface.

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Changes in expression of matrix metalloproteinases 1 and 8 in corneal epithelial cells after UV irradiation

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Purpose Matrix metalloproteinases 1 and 8 (MMP-1 and MMP-8) are collagenolytic enzymes that are associated with many ocular inflammatory diseases, such as uveitis, ulcerative keratitis, and ocular rosacea. It is suggested, that MMP-1 and MMP-8 are mainly produced by inflammatory cells so that an increased incidence of these enzymes in the cornea is associated with neutrophil infiltration. Based on our previous findings dealing with the coincidence of the expression of proteolytic enzymes (MMP-2 and MMP-9) with the severity of corneal injury induced by UV irradiation, the aim of this study was to investigate the effect of UVA and UVB rays on expression of MMP-1 and MMP-8 in the corneal epithelium.

Methods In the first group of rabbits the corneas were irradiated with UVA lamp (365 nm, once a day during 4 days, a dose per day 1.01 J/cm²), in the second group with UVB lamp (312 nm, once a day during 4 days, a dose per day 1.01 J/cm²). Normal corneas served as controls. MMP-1 and MMP-8 were examined in cryostat sections immunohistochemically using sheep polyclonal anti-MMP-1 and mouse monoclonal anti-MMP-8 antibodies.

Results Results show that UVA rays do not change expression of MMPs studied in corneal epithelium. In contrast, UVB rays induce overexpression of both MMPs in corneal epithelial cells.

Conclusion Comparing the effect of the same doses of UVA and UVB rays with the normal cornea, where the enzymes are only slightly (MMP-1) or moderately (MMP-8) pronounced in the epithelium, UVB rays (not UVA rays) evoked highly increased expression of both MMPs in irradiated corneal epithelial cells. The possible influence of overexpression of MMPs due to UVB-exposure on the development of corneal inflammation will be the aim of our next study.

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Trabecular meshwork contribution to the uveoscleral outflow

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Purpose To specify the anterior chamber source of uveoscleral outflow pathway.

Methods We performed perfusion of human cadaver eyes with India ink suspension through clear corneal tunnel. Lamellar scleral dissection revealed the ciliary muscle surface. We observed the character and volume of suprachoroidal fluid. After formaldehyde fixation we performed further dissection of trabecular meshwork and ciliary muscle with subsequent preparation of flat specimens of different layers of trabecular meshwork as well as histological sections for light microscopy.

Results Voluminous flow of India inc-stained fluid was observed after the ciliary muscle exposure. No unintended damage of the anterior chamber structures or choroid was found. Histological research revealed numerous ink particles in the trabecular meshwork and between the ciliary muscle bundles. The ink particles go from the anterior chamber to the Schlemm's canal and particularly retain at the scleral spur. The main stream goes along the trabecular beams of uveal layers (2-nd and 3-rd of 4 trabecular meshwork layers), bypass the scleral spur and flows into the ciliary muscle. No ink particles were found in the iris root.

Conclusion As the uveal trabeculae continue directly into the ciliary muscle bundles, the intertrabecular slits naturally continue into the spaces between the bundles of ciliary muscle. These slits may represent sufficient pathway for uveoscleral outflow. In the sclero-uveal layer aqueous humor can flow towards the ciliary muscle down to the scleral spur but not beyond. In the uveal layer the fluid can freely pass into radial portion of ciliary muscle. The other structures of the anterior segment appear to play no substantial role in the aqueous outflow.

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Cell connections between lens fibres in the posterior pole of human and bovine lenses

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Purpose Gap junctions (GJs) between lens epithelial cells (LECs) and between lens fibres (LFs) are prominent features of vertebrate lenses. However, they are absent between LECs and LFs. There is evidence for the presence of tight junctions (TJs) between LECs, and differentiating LFs but only in the equatorial pole. TJs are lacking between LFs. We are not aware of studies on cell-to-cell junctions in the posterior pole although its surface is about as large as that of the anterior surface. This study aimed to find morphological evidence for GJs & TJs in the posterior pole of bovine and human lenses using freeze fracture (FF) and thin section EM (TEM). It appeared that posterior LF membranes have a high density of intramembranous particles (IMPs). Broken necks of caveolae are sparse. Edge protrusions are often demarcated by single rows of IMPs. GJs are numerous on all LF membranes except on the outer, capsule facing, membranes. This suggests close coupling of posterior fibres. TJs proved to be totally lacking on all fibre membranes. TEM corroborated these findings, showing numerous GJs between posterior fibres. No evidence for TJs was found. However, numerous spot desmosomes were observed between the peripheral fibres.

Conclusion Lens fibres in the posterior pole are well coupled by GJs, but the presence of TJs could not be verified. The presence of spot desmosomes suggests a close mechanical attachment of posterior lens fibres. How the posterior pole regulates its solute transfer with the vitreous still remains to be elucidated.

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Macroglial changes in ipsilateral and contralateral rat retina in experimental glaucoma

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Purpose To investigate the effects of laser photocoagulation-induced moderate ocular hypertension (OHT), on the macroglia of the ipsi- and contralateral adult rat retinas at 3 weeks

Methods OHT in the left eye was chronically induced by diode laser photocoagulation of the trabecular meshwork and the perilimbal and episcleral veins. Animals were divided in three groups of study: Control (G0), age-matched untreated animals. Group 1 (G1), laser treated eyes which were further subdivided into two subgroups depending on the differences of averaged IOP with respect IOP levels of the contralateral eye, as follows: IOP difference lesser than 4 mmHg (G1A) and IOP difference greater than 4 mmHg (G1B). Retinal wholemounts were immunostained for GFAP. The retinal area occupied by astrocytes was determined and analyzed statistically.

Results In comparison with the eyes of age-matched untreated animals, the area of the retina occupied by astrocytes in the contralateral (untreated) eye was significantly reduced independently of the IOP level reached by the ipsilateral treated eye. The analysis of the area occupied by astrocytes in eyes with OHT revealed that there was a reduction of astrocytes significantly greater in G1B than in G1A with respect to the contralateral eyes. Müller glial cells were undetected for GFAP staining in eyes of age-matched untreated animals. However, in both treated and contralateral eyes Müller glial cells exhibited GFAP+immunoreactivity

Conclusion At three weeks of moderate OHT, the Müller cells of the adult retina exhibited a GFAP+immunoreactivity and a reduction of the retinal area occupied by astrocytes both in the treated and contralateral eye

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Human anterior lens capsule epithelial cell contractions

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Purpose The transport over the lens epithelial cell layer is governed by different morphological parameters and junctions whose defects can lead to cataract formation. The cells are cuboidal, tightly packed with very little intercellular space. We studied their contractions due to agonist application.

Methods Entire anterior human capsules obtained from cataract surgery, with the cells still attached, were used. A good part of the cell contacts, including contacts to the basement membrane were preserved. To measure the $[Ca^{2+}]_i$ and to monitor the cell morphology, the capsules were stained with the fluorescent dye Fura2. We applied either the agonist acetylcholine (ACh) solution or the physiological saline alone, to test whether the agonist is necessary to induce the cell contraction.

Results Lens epithelial cells in 23% (12/51) of the capsules contracted substantially upon ACh application and responded with an increase in $[Ca^{2+}]_i$. However, contraction also occurred, with very small increases in $[Ca^{2+}]_i$, when, as a control, physiological solution was applied in the same way, suggesting the possibility that the contraction can be induced mechanically.

Conclusion The major role of lens capsule epithelial cells is to be a regulating "barrier" between the aqueous humour and the lens fibre cells. The appearance of holes in this barrier, due to cell contraction, in response to not only agonist application but also presumably mechanical stimuli, compromises their basic function and may be a part of a pathophysiological mechanism associated with cataract formation.

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The human choroid possesses substance P and calcitonine gene-related peptide intrinsic neurons

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Purpose To determine the presence of substance P (SP)- and calcitonine gene-related peptide (CGRP) positive intrinsic choroidal neurons (ICNs) in human

Methods Nineteen choroidal whole-mounts were processed for indirect immunofluorescence. Antibody to a component of the neuronal cytoskeleton, neurofilament 200 kDa (NF-200), was combined with antibodies to SP and to CGRP (neuropeptides proper to the sensory nervous system).

Results The human choroid possesses numerous SP(+) and CGRP(+) ICNs. These neurons were observed in the suprachoroid, both in isolation and forming microganglia. For both types of ICNs studied, neurons were more numerous in the temporal than in the nasal regions. In both locations, SP(+) and CGRP(+) ICNs were more abundant in the central choroid (the choroid underneath the macular area of the retina), with cell density diminishing outwards to the choroidal periphery. There were no appreciable differences between the two populations of ICNs studied in terms of size, morphology or immunostaining characteristics.

Conclusion The human choroid contains an abundant population of SP(+) and CGRP(+) ICNs. Given that peripheral sensory innervation could be involved in the regulation of both choroidal blood flow and vascular architecture, the SP(+) and CGRP(+) ICNs described for first time in the present work may be implicated in these mechanisms of vascular regulation

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Pleiotropic effects of statins on the morphology of choroidal endothelial cells and vascular smooth muscle cells in hypercholesterolemic rabbits

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Purpose To describe the ultrastructural changes in the choroid of long-term hypercholesterolemic rabbits after a low dose statin treatment and to evaluate some pleiotropic effects of these drugs on the morphology of endothelial cells (EC) and vascular smooth muscle cells (VSMC).

Methods New Zealand rabbits were divided into three groups: G0, fed a standard diet; G1, fed a 0.5% cholesterol-enriched diet for 8 months; and G2, fed a 0.5% cholesterol-enriched diet for 8 months plus administration of fluvastatin sodium or pravastatin sodium at a dose of 2 mg/Kg/day each. Eyes were processed for transmission-electron microscopy.

Results G1 had a build-up of lipids at the suprachoroida that compressed the vascular layers with the lumens of the vessels to the point of collapse in some instances. In contrast, G2 had a substantially decreased number of suprachoroidal foam cells and of lipids in the vascular layers and the vascular lumens were normal. The preservation of cytoplasmic organelles, caveolar system and other ultrastructural features of EC and VSMC in G2 was in contrast to the numerous signs of necrosis observed in G1. Bruch's membrane in G2 contained fewer lipids and more collagen than in G1.

Conclusion Treatment with a low dose of fluvastatin sodium or pravastatin sodium reduced the build-up of lipids and the macrophages in the choroid and restores the vascular lumens of choroidal vessels independently of the cholesterol effect. The normal ultrastructural features of choroidal EC and VSMC in statins treated animals suggest that the endothelial function is preserved and the ischemia reduced.

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Proteasome inhibitor -induced protein aggregation is regulated via HSP90, HDACs and microtubulus stability in ARPE-19 cells

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Purpose Impaired degradation of cellular proteins is implicated in aged RPE cells. In addition to lysosomal protein clearance, many cellular proteins are degraded in proteasomes. Heat shock proteins (HSPs) tend to prevent the accumulation of cytotoxic protein aggregates. Regulatory role of HSPs, histone deacetylases (HDACs) and tubulin stability state in proteasome inhibitor -induced protein aggregation in ARPE-19 cells were evaluated.

Methods HSP90, HSP70, HSC70, HSP27, ubiquitin and acetylated tubulin expression levels were analyzed by Western blotting. Phase contrast and transmission electron microscopy and immunofluorescence analysis were used to detect cellular organelles and to evaluate morphological changes.

Results Western blotting analysis showed increased HSP70 expression levels in response to HSP90 inhibitor geldanamycin (GA) and proteasome inhibitor MG-132. Trichostatin A (HDAC inhibitor) and taxol evoked increased acetylation level of tubulin. Interestingly, MG-132 -induced juxtanuclear protein aggregates were not formed in response to GA, while during taxol treatment aggregation process was conventional. During trichostatin A and nocodazole treatment the aggregates were localized to periphery of cytoplasm rather than to juxtanuclear position. The HSP90 could not be seen together with the aggregates, while the HSP70 stained strongly with the observed juxtanuclear aggregates.

Conclusion HSP90, HDACs and tubulin polymerization state are critical regulators of proteasome inhibitor -induced protein aggregates in ARPE-19 cells. However, total acetylation level of tubulin does not seem to be essential in the aggregation process.

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Retinal ganglion cells in culture induced to die by insults of hydrogen peroxide (H₂O₂), rotenone and glutamate/buthionine sulfoximine (GB) are differentially protected by the flavonoids, epicatechin gallate (EG) epigallocatechin gallate (EGCG) and genistein

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Purpose To investigate the neuroprotective properties of three different flavonoids.

Methods: Immortalized retinal ganglion cells (RGC-5 cells) in culture were exposed H₂O₂, rotenone or GB in the presence or absence of EG, EGCG or genistein and cultures analysed by viability assays, immunocytochemistry, western blot, stimulation of reactive oxygen species (ROS) and for histological evidence for apoptosis.

Results Insults of H₂O₂, rotenone and GB resulted in a time and dose-dependent stimulation of ROS associated with an apoptotic type of cell death of RGC-5 cells. Importantly, EG blunted these effects significantly but genistein was ineffective. In contrast, EGCG attenuated these effects only for insults induced by H₂O₂ and rotenone and not by GB.

Conclusion The results suggest the idea that defined insults can be attenuated by specific flavonoids.

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HSP70 and p62/SQSTM1 regulate differently autophagy clearance in ARPE-19 cells

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Purpose Prior to proteolysis, heat shock proteins (HSPs) attempt to refold misfolded proteins. If this process is not successful proteins are degraded in proteasomes or in lysosomes. In the present study, the roles of the HSP70 and the p62/SQSTM1 in autophagy clearance were evaluated in ARPE-19 cells after proteasome inhibitor treatment.

Methods The HSP70, p62/SQSTM1 and ubiquitin localization were analyzed by immunofluorescence. Confocal and transmission electron microscopy were used to detect cellular organelles and to analyze the morphological changes. HSP70 and p62/SQSTM1 levels were modulated using RNA interference techniques. Cell viability was measured by colorimetric assay.

Results The proteasome inhibitor MG-132 evoked the accumulation of perinuclear aggregates positive for HSP70, p62/SQSTM1 and ubiquitin-protein conjugates. We observed that the aggregation was reversible: a cessation of proteasome inhibition led to clearance of the deposits via autophagy. Interestingly, p62/SQSTM1 mRNA depletion delayed autophagy clearance and significantly increased cell death in conjunction with proteasome inhibition. The hsp70 RNA interference did not change autophagy clearance, but increased cell death in response to proteasome inhibition.

Conclusion The HSP70 and p62/SQSTM1 regulate differently autophagy clearance in RPE cells. Autophagy seems to be an important mechanism to clean proteasome inhibitor -induced protein aggregates.

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Human retinal pigment epithelium expressed tight junction markers in spheroid cultures

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Purpose To determine if spheroid cultures generated from human prenatal retinal pigment epithelium (RPE) express markers for mature RPE and tight-junction proteins and secrete components of the Bruchs membrane.

Methods RPE was dissected from human prenatal donor eyes, chopped into 200 µm sections and place in suspension culture in serum-free defined medium containing the commercially formulated supplement B27. Cultures were grown as spherical aggregate suspensions. At various times darkly spheroids were removed from the suspension and fixed with 4% PFA for one hour and cryoprotected with sequential sucrose solution. The spheroids were embedded in OCT, cut in 16 µm cryosections, and stained for different RPE and membrane markers including RPE65, CRALBP, PEDF, Occludin, Ezrin, Zo-1 and laminin. EM was also performed.

Results Inside the spheroids, RPE cells were located around a central space containing secreted laminin without a clear structure. Cells expressed tight-junctions markers including ZO-1, ezrin and occludin. Some of the cells had intracytoplasmic expression on CRALBP, RPE65 and PEDF. Long term culture spheroids showed a decrease in the number of RPE cells and increased pigment and debris. When examined with EM, RPE cells were located over a layer of fibroblasts, showing microvilli towards the outside of the spheroid and intracellular pigment.

Conclusion Prenatal RPE maintained as spheroids show some RPE mature markers expression as well as tight junction markers. The cells are able to secrete laminin, an important component of the Bruch membrane.

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A new potential pharmacological target in diabetic retinopathy: the HuR pathway

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Purpose A key player within diabetic retinopathy is the Vascular Endothelial Growth Factor (VEGF) whose expression seems under Protein Kinase C (PKC) control, although no data are available on the molecular pathway underlying this process. Our previous in vitro work in retinal bovine pericytes (Amadio et al., *Pharmacol Res.* 57: 60, 2008) described a new molecular cascade involving PKCβ, the mRNA-stabilizing protein HuR and VEGF. We investigated whether this pathway is operant also in vivo and altered by diabetes.

Methods After 10 days treatment, retinal tissues from streptozotocin (STZ)-induced diabetic rats were processed to detect PKCβI and βII, HuR and VEGF content. HuR activation/phosphorylation was also investigated. Immunoprecipitation coupled to real-time quantitative PCR was employed to evaluate HuR binding to VEGF mRNA in ribonucleoprotein complexes (mRNP). The specificity of the PKCβ involvement was tested by experiments performed with a selective PKCβ inhibitor.

Results In retinal tissues from STZ-induced diabetic rats, PKCβI (+160%, p<0.01) and PKCβII (+113%, p<0.05) levels were increased versus sham. In the same STZ tissues a PKC-mediated phosphorylation of HuR (+209%, p<0.01) occurred. A specific binding between HuR protein and VEGF mRNA was also detected in retinal mRNP. As well, the PKCβ/HuR activation was accompanied by enhanced VEGF protein expression (+256%, p<0.05). All these effects were blunted by a selective PKCβ inhibitor.

Conclusion These findings first demonstrate the existence of the PKCβ/HuR/VEGF pathway in experimental diabetic retinopathy, suggesting that this cascade may represent a potential pharmacological target to counteract diabetic retinopathy, and more generally pathologies implicating VEGF deregulation.

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Inhibition of Notch pathway enhances photoreceptor commitment from cultured retinal stem cells

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Purpose Consequently to the principle that photoreceptors have to be at a very precise development stage to be successfully transplanted (MacLaren 2006), we are trying to mimic this development stage in vitro using retinal stem cells. The latter were isolated from the newborn mouse retina, derived from the radial glia population, and were previously isolated and characterized in our laboratory. We developed a protocol to commit these cells to the photoreceptor fate, but even if the percentage of cells expressing photoreceptor markers is high (30%), the differentiation process is incomplete so far (Merhi-Soussi 2006).

Methods To ameliorate photoreceptor differentiation, we hypothesized that the Notch pathway may interfere with this process by either promoting glia commitment, or maintaining an undifferentiated state. We are thus using a gamma-secretase inhibitor (DAPT), which inhibits Notch activation, during the in vitro differentiation process.

Results The constant presence of DAPT i) leads to a 233% increase in peripherin/RDS-positive (photoreceptor marker) cells, compared to controls (no DAPT, n=3, P<0.02) along with a 68% decrease in GFAP- positive cells (n=3, P<0.04), ii) modifies the ratio between uni-/bi- (23%) and multi- (77%) polar peripherin/RDS-positive cells to 45% and 55%, respectively, and iii) reduces by 50% the total cell number during the whole differentiation process.

Conclusion We are now exploring whether this reduction in total cell number is due to inhibition of cell proliferation or to cell death and whether photoreceptor differentiation is promoted instead of glial induction. Such protocol may help to better mimic photoreceptor development, but this needs to be confirmed by genomic and proteomic profile analyses.

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Rarebit visual field follow-up in pediatric glaucoma

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Purpose To evaluate the long term change of the Rarebit (RB) visual fields and optic disc images in a group of children, maximally treated for paediatric glaucoma of various origin and severity, and compare them to normal RB visual field development during adolescence.

Methods Thirteen subjects (24 eyes) with pediatric glaucoma and 14 control subjects from the total group of 15 glaucoma and 15 control subjects in a previous study were included in the current follow-up study. For comparison, RB visual field data from 4 other studies including healthy children were used. Data regarding best corrected visual acuity, refraction (spherical equivalent), intra-ocular pressure, optic nerve appearance, diagnosis and treatment in the glaucoma group was collected from the medical records. In the control children, best corrected visual acuity was measured after determination of the refractive errors using an autorefractor. All participating subjects underwent RB visual field test and optic disc topography measurements using the Heidelberg Retina Tomograph.

Results A majority of both glaucoma eyes and control eyes showed visual field improvement. Using the same limit of normality as in the previous study (MHR above 90% and less than 5 depressed locations), 6 out of the 23 RB visual fields, previously classified as pathological, were now classified as normal. Some visual fields, both in the control and the glaucoma group showed slight deterioration, not leading to re-classification. Twenty-one of 26 eyes in the glaucoma group were examined with both RBP and HRT3. The concordance between the methods was 0.6 (Cohen's kappa).

Conclusion In adolescents, maximally treated for glaucoma, both the optic nerve heads and the visual fields remain stable during 5 years of follow-up.

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The retinal nerve fiber layer and the optic nerve head morphology after glaucoma surgery

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Purpose To detect and quantify changes in the retinal nerve fiber layer (RNFL) and the optic nerve head (ONH) morphology after glaucoma surgery.

Methods 13 eyes of 13 patients with open-angle glaucoma in which goniotrephining with scleral flap without intraoperative antimetabolites for progressive glaucoma damage was done were included in this prospective study. Before and 6 months after the surgery: the intraocular pressure (IOP) was measured, the thickness of the RNFL was measured with a scanning laser polarimeter (GDx VCC), the confocal scanning laser ophthalmoscopy measurements of ONH with Heidelberg retina tomograph (HRT 3) were performed and the visual field was tested with Humphrey Field Analyser.

Results The mean IOP before surgery was 24.5 ± 2.3 mmHg decreasing 6 months after to a mean of 13.9 ± 3.0 mmHg ($p < 0.05$). The RNFL measurements with GDx VCC revealed no differences between the mean TSNIT Average ($p = 0.383$), mean Superior Average ($p = 0.756$) and mean Inferior Average ($p = 0.269$) before and after surgery. The ONH measurements with HRT 3 revealed postoperatively a significant increase in the mean Rim Area, Rim Volume and Cup Shape Measure, whereas Cup Area, Cup Volume and Linear Cup/Disc Ratio decreased ($p < 0.05$). There were no differences between the mean Height Variation Contour ($p = 0.678$) and Mean RNFL Thickness ($p = 0.064$) before and after surgery. Preoperatively the mean value of the Mean Deviation on automated perimetry was -18.82 ± 8.5 dB improving 6 months postoperatively to a mean of -16.63 ± 7.9 dB ($p < 0.05$).

Conclusion Our study demonstrated the beneficial effect of IOP reduction obtained with glaucoma surgery on visual field indices and ONH parameters evaluated by HRT 3.

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Sympathetic influence on intraocular pressure

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Purpose To apply wavelet analysis to investigate the sympathetic modulation upon intraocular pressure (IOP) of the cold pressure test

Methods In an autonomic dedicated lab continuous and simultaneous recording of (IOP) with Pascal (DCT) and arterial blood pressure (BP) were made in 11 healthy individuals, of both sexes, in basal conditions and during 1 minute of cold-pressure test (CPT). The tests were in the morning period and in the 24h previous individuals were told not to smoke and to avoid xanthines or other stimulants ingestion. Individuals under medication or with other pathology that could affect the autonomic nervous system were excluded. Data analysis of IOP and systolic BP (SBP) in the time-scale domain with wavelets (DB12) was performed, for both variables, during the last minute prior to test and during one minute upon test. In this last case, periods of 10s were individually analysed and the period with the largest value was the chosen for statistical analysis using t-Student test, Welch corrected (Ducla-Soares et al, 2007). LF IOP and LF SBP were calculated and differences between basal and CPT values were considered significant where $p < 0.05$. Data were expressed as mean \pm SEM, and for LF mmHg²

Results Data refer to 6 male and 5 female with a mean age of 49 [40-70]. On CPT, a sympathetic challenging manoeuvre, an increase of SBP was observed. Wavelet analysis of both signals showed a significant increase of LF IOP and LF SBP from 0.13 ± 0.15 and 8.66 ± 1.51 to 0.37 ± 0.26 mmHg² and 18.7 ± 4.5 mmHg², respectively, and a not significant change in IOP 17.0 ± 2.6 to 18.0 ± 1.5

Conclusion Our data show a sympathetic signature on IOP correlated with that observed in BP. We show that wavelets are a suitable tool for the evaluation of sympathetic signature on short periods of IOP recorded by DCT

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Vascular endothelial dysfunction in primary open angle glaucoma

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Purpose Glaucomatous optic neuropathy (GON) is related not only to elevated intraocular pressure (IOP) but also to an insufficient ocular blood supply related to a vascular dysregulation. We evaluated peripheral vascular endothelial function in primary open-angle glaucoma (POAG) with a non-invasive endothelium flow mediated vasodilatation (FMD).

Methods We studied 20 POAG patients. The diagnostic criteria for POAG was an IOP exceeded 22 mm/hg before treatment, open anterior chamber angle, cup/disc ratio > 0.7 and optic nerve related visual field loss (MD > 6 dB, SF > 2.5 dB and CPSD > 3 dB). Patients with systemic diseases such hypertension, heart failure and diabetes mellitus were excluded. All patients and 20 normal controls underwent measurement of FMD with high-resolution 2-dimensional ultrasonographic imaging of the brachial artery by a Philips ENVISOR echographic machine. To induce iperemia a sphygmomanometer cuff was inflated to 250 mm/hg for 5 minutes. The cuff then was deflated rapidly and at 60 seconds after cuff deflation 2-D images of the brachial artery were recorded for 5 seconds. A statistical analysis was made of the FMD in POAG and in normal control patients using the Student "t" test for unpaired data and the Pearson correlation test to show the correlation between the endothelial dysfunction and the visual field indexes.

Results Patients affected by POAG exhibit lower values of FMD compared with healthy people. In POAG patients we found an FMD 4.28 ± 2.49 vs. 8.66 ± 3.02 ($p < 0.001$). The mean decrease (MD) visual field index was related to FMD ($p < 0.05$).

Conclusion Patients with POAG have an impaired vascular function related to a vascular endothelium dysfunction and the MD visual field is related to FMD impairment.

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Sympathetic variability of intraocular pressure of glaucoma patients evaluated by wavelets

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Purpose We had showed the usefulness of wavelets in the detection of sympathetic activity in intraocular pressure (IOP). With we intend to evaluate sympathetic activity in glaucoma patients with and without topical therapeutics through the same methodology

Methods 3 groups of individuals of both sexes: 1) a healthy individuals (HI, n=11); 2) two groups of glaucoma patients with Primary Open-Angle Glaucoma, one under treatment (GT, n=25) and another without (GNT, n=10). In an autonomic Lab. Continuous recording of IOP with Pascal-DCT, in day before, individuals were told not to smoke and to avoid xanthines or other stimulants ingestion. Glaucoma patients were diagnosed by visual fields defects and optic disc analysis. For GT patients a wash-out time of 1-3 weeks was required depending on therapeutics. Individuals under medication or any other pathology that could affect the autonomic nervous system were excluded. Data analysis of systolic IOP in the time-scale domain with wavelets (Db12) was performed during one minute. Statistical analysis using t-Student, Welch corrected (Ducla-Soares et al, 2007) was used. LF IOP was calculated for the 3 groups and differences were considered significant where $p < 0.05$. Data (mean \pm SEM)

Results Mean age 49 (40-70), 64 (45-67) and 61 (36-61) for HI, GT and GNT groups, respectively. The quantization of sympathetic variability of SIOP by wavelets was 0.4 ± 0.17 , 0.122 ± 0.075 and 0.118 ± 0.067 for HI, GT and GNT groups respectively. Values were significantly different between HI and GT ($p < 0.05$) and GNT ($p < 0.05$) but not between the two groups of glaucoma patients

Conclusion Our data show that glaucoma patients have a significant decrease in sympathetic activity that could be due to an extraocular mechanism that persists with therapeutics

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Measurement of IOP with radiowave telemetry



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Purpose To illustrate the implantation technique & tolerance of a novel wireless silicone-encased intraocular pressure transducer in rabbit eyes. The device can conceivably provide a reliable way to measure the IOP in situations where measurement is difficult with the present technology, for instance in eyes with keratoprosthesis implants.

Methods The transducer, manufactured by Mesotec GmbH (Hannover, Germany), is a fully digital ultra miniature system, integrating pressure sensing, data handling and telemetry on a single microchip. The microchip is connected to a telemetry coil and requires no internal power source. The data is received using an external reader unit. The transducers were initially tested for calibration in enucleated bovine eyes. They were sterilized with ethylene oxide. They were implanted either through a corneal autograft (n=2) or through a large limbal incision (n=2) and placed either in the sulcus (n=2) or suspended into the vitreous cavity (n=2) after removing the crystalline lens. In one rabbit (SHAM), the crystalline lens was removed but no implant was placed. The eyes were closed with interrupted 10-0 nylon sutures. Daily observations and weekly full clinical examinations were performed. The readings obtained by the transducer-readers were compared with those obtained by Tonopen.

Results The transducers were well tolerated, with minimal transient post-operative intraocular inflammation that was similar in both the experimental and SHAM groups at comparable time-points. The pressure obtained by the transducers showed good correlation with those obtained by Tonopen.

Conclusion Our animal studies show that the transducer can be easily implanted and is well tolerated inside the rabbit eye, up to 4 months at the present time.

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Correlation between Goldmann applanation tonometry and rebound tonometry in relation to central corneal thickness

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Purpose To investigate the influence of central corneal thickness (CCT) on the ICare Rebound Tonometer (RBT) measurements and to verify the correlation of RBT and Goldmann applanation tonometry (GAT).

Methods The current study contains data from 230 eyes from a mixed population of the outpatient departments of the University Eye Clinic Salzburg. The mean-age was 67.19 years (SD +/- 11.87). The study was explained to the patients and their informed consent was obtained. The IOP was measured with both the GAT and the ICare-tonometer; in addition the CCT was determined with standard pachymetry.

Results We were able to show a good linear correlation between GAT and RBT readings (r=0.9). Over the whole range of IOP we could observe a good correlation of both tonometers with the tendency of a slight overestimation of the IOP in RBT readings. CCT has a stronger impact on the results of RBT than on the results of GAT. In thin corneas RBT tends to underestimate the GAT readings, whereas in thick corneas RBT overestimates the GAT readings. Although the correlation factor between GAT and RBT is high, significant differences can be seen in the Bland-Altman analysis. Only in 15% of all measurements no differences in results between the two methods were observed. In 26% the difference was 1 mmHg, in 23% 2 mmHg, in 19% 3 mmHg and in 16% 4 or more mmHg. The analysis also revealed a correlation of r=-0.4 between CCT and the difference in results of the two methods.

Conclusion In cases where an exact measurement of the IOP is important RBT would not be the first choice. Also formulas to correct for the dependency of RBT on CCT would improve the results only marginally.

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Factors affecting ocular rigidity in normal human eyes

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Purpose To measure the ocular rigidity coefficient and evaluate its relation with axial length (AL), age and mean systemic blood pressure (SBP).

Methods Sixty three patients (63 eyes) undergoing cataract surgery, with different refractive errors and no ocular or systemic pathology were enrolled in this study. An invasive, computer controlled device comprising a microdosimetric pump and a pressure sensor, is connected to the anterior chamber under topical anaesthesia with drops. The system is used to raise the intraocular pressure (IOP) from 15 to 40mmHg, by infusing the eye with a saline solution. After each 4 ul infusion step, the IOP is continuously recorded for 2 seconds. From an initial level of 40mmHg an IOP decay curve of 1 minute is obtained. SBP and pulse rate are measured during the procedure. The rigidity coefficient is calculated by an exponential fit to the pressure volume data after correction for outflow. The study was approved by the Institutional Board and performed under the patient's informed consent.

Results Mean AL was 24.8 (range 21.2-32.5). Mean age and SBP was 59 (12) years and 93.7 (10.5) mmHg respectively. The mean ocular rigidity coefficient was 0.021 (0.005) ul-1. Increasing axial length is associated with a decrease in the rigidity coefficient (r=-0.61, p<0.01). A positive correlation between the rigidity coefficient and age of the patients is found (r=0.31, p=0.01), whereas similar findings were not observed for SBP (p>0.05).

Conclusion This manometric approach of measuring ocular rigidity provides a normative database of this parameter in living human eyes. Axial length and age influence ocular rigidity. These results may have implications on tonography and ocular pulse studies.

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On the dependence of outflow facility on Intraocular pressure in the living human eye.

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Purpose The change of outflow facility is determined by the value of intraocular pressure (IOP) possibly due to changes in the angle of the anterior chamber, the trabecular meshwork and the Schlemm's canal. The purpose of this study was to determine the relationship between outflow facility and IOP (especially for elevated values of IOPs) in living human eyes.

Methods Thirteen eyes from thirteen cataract patients (6 men and 7 women, aged 61 years, sd 15) were enrolled in the study. The study was approved by the Institutional Review Board. The measurements were performed before cataract surgery, using an intraoperative invasive manometric device. The pressure-time decay was recorded in order to calculate the outflow facility at different values of IOP. An appropriate mathematical model was developed to calculate outflow facility based on the IOP-time curve and the ocular rigidity of each eye under measurement.

Results The average outflow facility coefficient was 0.213 (SD 0.097) µl/min/mmHg. Our data suggest that outflow facility has a non-linear relationship with IOP, especially in the range of higher IOPs. This non-linear behavior of outflow facility was approximated with an exponential function. There was no significant statistical correlation (r=0.2377, p=0.4568) with outflow facility and ocular rigidity.

Conclusion Outflow facility depends on IOP in a non-linear manner that indicates a complex relationship between IOP and the geometry of the outflow pathways. In this limited set of measurements, a relationship between the outflow facility (or its non-linearity) with other parameters could not be established.

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IOP evaluation in glaucoma patients after phacoemulsification

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Purpose To evaluate intraocular pressure in glaucoma patients after cataract surgery with phacoemulsification

Methods 107 glaucoma patients underwent phacoemulsification cataract extraction from June 2006 to May 2009. The follow up period was 6 months. No medication was discontinued preoperatively. The mean value of preoperative IOP was 16.33 mmHg (10-28mmHg). 41 patients (38.3%) were under single medication treatment, 35 (32.7%) were under two medications, 23 (21.5%) were under three and 8 (7.5%) were under four medications. IOP was measured on the 1st day, 1 week, 1, 2, 3 and 6 months postoperatively. If IOP was <25mmHg, no additional medication was given, and if IOP was >25mmHg, acetazolamide was given during the first week and topical medication after that

Results Final IOP was <20mmHg without treatment in 50.8% of the patients, 27.6% were under single medication treatment, 17.9% were under two medications, 3.7% were under three medications and none was under four medications

Conclusion A reduction of at least one glaucoma medication was observed postoperatively. Cataract extraction with phacoemulsification has a positive influence in postoperative IOP in glaucoma patients and can delay or even avert a possible trabeculectomy.

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SITA-SWAP and SITA-SAP in glaucoma suspects

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Purpose To compare the ability of Standard and Short-Wavelength Automated Perimetry to point out early functional defects in glaucoma suspects picked up using imaging tests such as GDx-VCC and HRT-III

Methods Randomly selected 90 eyes of 90 patients screened on the basis of clinical suspicion of having early glaucoma, were submitted to a complete ophthalmic examination including GDx and HRT. Moorfields regression analysis (MRA), cup shape measure (CSM), rim area (RA) for HRT-III and nerve fiber indicator (NFI), superior and inferior TSNIT average for GDx-VCC were taken into account. If at least one of the parameters was found out of the normal limits, a 24-2 SITA-SAP and SITA-SWAP tests. Mean defect (MD), pattern standard deviation (PSD), number of points significantly depressed <5% and <1% in the pattern deviation plot and test duration for SAP and SWAP were compared using the Wilcoxon signed rank test and Bland and Altman plot. Media opacities >1 according to LOCS-III were excluded

Results According to the results of the Wilcoxon test SWAP gave a significantly larger MD and PSD defect than SAP (p<0.01) and a larger number of depressed points with p<1% (p<0.01). SWAP test duration was shorter than SAP (p<0.01). These results were not confirmed by Bland and Altman plot that could not point out significant differences between the parameters provided by SWAP and SAP

Conclusion In patients with suspicion of early glaucomatous damage given by imaging techniques (GDx-VCC and HRT-III), SITA-SWAP is not able to identify a functional loss in all cases. The SITA applied to SWAP can improve the ability of the perimetric test to pick up early defects involving short-wavelength sensitive ganglion cells at least in some patients and in a shorter test time than SITA-SAP

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Functional correlation between retinal sensitivity threshold values of standard automated perimetry in glaucoma

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Purpose To study the interrelationship among the 52 retinal sensitivity threshold values of the Humphrey visual field analyzer with the 24-2 Swedish interactive threshold algorithm (SITA) standard strategy in glaucoma patients.

Methods Prospective cross-sectional study. 104 eyes of 104 patients with diagnosed glaucoma by means of optic disc stereophotographs were evaluated. Each 52 threshold points of the SITA Standard 24.2 program in the standard automated perimetry (SAP) were obtained. The upper and the lower hemifield were studied separately. Pearson correlation coefficients were calculated between each of the mean threshold values at each point of the visual hemifield and the rest of the threshold points.

Results Perimetric correlation maps between threshold values in the same hemifield were obtained. Most of the points had moderate and high correlations ($r \geq 0.65$) with neighboring points and distant points corresponding to the retinotopic distribution of the retinal nerve fibers.

Conclusion There is a functional relationship, in glaucoma patients SAP, between neighboring and distant threshold points in corresponding to the anatomical arrangement of ganglion cells axons. This enables to achieve perimetric patterns of glaucoma defects.

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Relationship between frequency-doubling perimetry indices and optic nerve head parameters measured with the Heidelberg retina tomograph 3

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Purpose To determine the relationship between the indices of frequency-doubling perimetry (FDT) and the optic disc parameters measured with the Heidelberg Retina Tomograph (HRT) 3 in glaucoma patients.

Methods 74 eyes of 74 glaucoma patients were prospective and consecutively selected. All of them had intraocular pressure higher than 21 mmHg, and reproducible glaucomatous visual field loss in standard automated perimetry. FDTs were performed with a Humphrey FDT perimeter using the C-20-5 full threshold strategy. Participants underwent imaging of the optic disc with the HRT3. The Kolmogorov Smirnov test was applied to check the data were normally distributed. Pearson correlations were calculated between FDT indices (mean deviation and pattern standard deviation) and global stereometric HRT3 parameters.

Results Mild to moderate significant correlations (p<0.05) were observed between the FDT indices and most HRT3 parameters. The strongest correlation was found between the mean deviation of FDT and FSM discriminant function of HRT3 (0.525). Pattern standard deviation had the strongest correlation with rim area (-0.431).

Conclusion Optic nerve head parameters measured with HRT3 showed reasonable agreement with FDT indices in glaucoma patients.

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Relationship between the automated classification of Heidelberg retina tomograph and main indices of short-wavelength automated perimetry

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Purpose To evaluate the relationship between Glaucoma Probability Score (GPS) provided by the Heidelberg Retina Tomograph (HRT) version 3 and main indices of short-wavelength automated perimetry (SWAP) in glaucomatous eyes.

Methods A total of 66 glaucoma patients were included in the study. Eyes were classified depending on intraocular pressure and standard automated perimetry (SAP) results. Only one eye per patient was randomly chosen. All participants underwent imaging with the HRT3, and numerical values of GPS were included in the statistical analysis. The Kolmogorov Smirnov test was applied to check data were normally distributed. Pearson's correlations were calculated between global indices of SWAP (mean deviation [MD] and pattern standard deviation [PSD]) and global GPS values of HRT3.

Results Mean age was 54.75 ± 9.1. MD of SAP was -6.2 ± 6.1, and MD of SWAP was -7.1 ± 5.8. No significant (p<0.05) correlations were found between GPS values and main indices of SWAP.

Conclusion GPS evaluates glaucomatous damage independently of SWAP results.

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An analysis of retinal nerve fiber layer with ImageJ program

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Purpose So far retinal nerve fiber layer(RNFL)analysis in red-free fundus photograph has been qualitative. The purpose of this study is to introduce the gray scale of imageJ program as quantitative tool for evaluation of RNFL in red-free fundus photograph.

Methods Subjects were normal healthy 120 eyes of 120 patients aged 20-50 years old. They were recruited from referred glaucoma suspected patients from March 2008 to December 2008 at Kim's eye hospital. Refractive error above 6.0Diopter, large peripapillary atrophy, previous other ocular disease subjects were excluded. Red-free digital fundus photographs were taken by Kowa nonmyd7 fundus camera and Nikond70s digital camera by one experienced examiner with pupil dilatation. A circle was drawn around the optic disc nerve head(ONH)margin on Red-free digital fundus photographs. The ONH divided into 12 clock-hours sectors. Sectors run clockwise in the right eye and counter clockwise in the left eye. The gray scales of RNFL adjacent to ONH were calculated by three observers.

Results The gray scale of observer1 were calculated from 78+/-21.6 to 125+/-15.2. The gray scales were lowest at 3o/c and highest at 11o/c. The gray scale of observer2 were calculated from 87.8+/-20.7 to 128.6+/-13.5. The gray scales were lowest at 3o/c and highest at 7o/c. The gray scale of observer3 were calculated from 86.7+/-24.8 to 128.4+/-16.4. The gray scales were lowest at 3o/c and highest at 11o/c. Interclass correlation coefficient was from 0.864 to 0.985.

Conclusion The gray scales of RNFL were high in superotemporal and inferotemporal area and low in nasal area. The gray scale of imageJ showed high correlation between three observers. The gray scale of imageJ program can be objective tool for RNFL evaluation with red-free fundus photograph.

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Reproducibility of optic disc measurements acquired with spectral-domain optical coherence tomography

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Purpose To compare the reproducibility of peripapillary retinal nerve fiber layer (RNFL) measurements of 2 optical coherence tomography (OCT) systems.

Methods Fifty-seven healthy individuals were prospectively selected. Only one eye was randomly chosen. All participants had intraocular pressure less than 21 mmHg and normal standard automated perimetry. Peripapillary RNFL thicknesses were measured with the Cirrus (Carl Zeiss Meditec, Dublin, Ca) and Spectralis (Heidelberg Engineering, Heidelberg, Germany) OCTs. The same operator acquired 3 consecutive series of scans. Left eye data were converted to a right eye format. Intraclass correlation coefficient (ICC), coefficient of variation (COV), and test-retest variability were calculated for all parameters.

Results Mean age was 58.3±11.3 years. The ICC was higher than 0.92 for all parameters of both OCTs. The COV ranged from 1.8% (average thickness, Spectralis) and 2.5% (average thickness, Cirrus) to 5.8% (nasal inferior segment, Spectralis) and 6.4% (2 o'clock segment, Cirrus). Test-retest variability was lower than 12.2 µm for Cirrus parameters and lower than 10.9 µm for Spectralis parameters.

Conclusion Both OCT systems had similar reproducibility of peripapillary RNFL measurements.

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Differences in retinal nerve fiber layer measurements between time domain optical coherence tomograph (OCT) and spectral domain OCT in patients with type 1 diabetes without retinopathy

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Purpose To compare Retinal Nerve Fiber Layer measurements with Time Domain Optical Coherence Tomograph (OCT) and Spectral Domain OCT and to get a formula to extrapolate thickness results from time domain to spectral domain OCT.

Methods Thirty patients with Type 1 diabetes (DM) without retinopathy were prospective and consecutively studied. All underwent imaging of Retinal Nerve Fiber Layer with Stratus OCT (Carl Zeiss Meditec, Inc.)(Fast RNFL thickness scan strategy), Cirrus HD OCT (Carl Zeiss Meditec, Inc.)(optic disc scan strategy) and Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany)(RNFL scan strategy). RNFL measurements with the 3 devices were compared for the average thickness and for the four quadrants.

Results Mean RNFL average thickness was 100.02 ± 11.28 microns with Stratus OCT, 95.35 ± 8.38 µm with Cirrus OCT and 98.9 ± 8.92 µm using Spectralis OCT. All RNFL measurements show significant differences except nasal quadrant between cirrus and stratus, and temporal quadrant between stratus and spectralis. We found that Stratus average thickness is 4.67 microns larger than Cirrus, and 1.12 microns higher than Spectralis OCT.

Conclusion Retinal Nerve Fiber Layer measurements show differences between Stratus, Cirrus and Spectralis OCT in type 1 DM patients without retinopathy. The 3 device are not directly comparable.

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Glaucomatous optic neuropathy and oscillatory potentials in the ERG to long-duration stimuli

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Purpose To mark out OPs in the long-duration flash ERG in primary open angle glaucoma (POAG) and to determine their role in diagnosis and monitoring of glaucomatous optic neuropathy (GON).

Methods 20 healthy subjects and 45 patients with different stages of glaucoma were investigated. Standard OPs and ERG to long-duration stimuli (200 ms) were recorded. OPs for ON and OFF ERGs were isolated after mathematic filtration (80-200 Hz). Optic discs of patients were evaluated by confocal scanning laser ophthalmoscopy HRT II. Index (mean) of early and late standard OPs were calculated separately.

Results Marked oscillations were determined in both ON and OFF ERG to long-duration stimuli (3-4 and 2-3 waves respectively). Different decrease of amplitudes were detected depending on the stage of POAG. Subnormal OFF-response of ERG to long-duration stimulus and late standard OPs were observed in all stages of POAG. ON-response of ERG to long-duration stimulus and early standard OPs in the early stage of POAG were supernormal, whereas in the advanced and late stages they were subnormal. A statistically significant correlation was observed between standard OPs and OPs in the ERG to long-duration stimuli.

Conclusion In POAG the OFF cone channels damage earlier. OPs of ERG to long-duration stimulus are more sensitive indicator of ischemic processes in the retina. A strict correlation between different components of ERG may suppose that early waves of standard OPs are generated by ON-components of cone and rod pathways, while the origin of OFF-OPs is related to OFF-components of cone pathway.

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Is subconjunctival inflammation associated with failure of filtering surgeries?

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Purpose To investigate if inflammatory conjunctival reaction associate with antiglaucoma treatment, compromises the results of filtering surgeries.

Methods Histological sections of conjunctival specimens obtaining from 35 eyes, during surgical procedure, were fixed in glass slides, prepared and submitted to immunohistochemical reaction for detection of HLA-DR marker (presence of brown staining close the cell membrane).

Results The presence of inflammation was detected in specimens from 45.7% of the eyes. The rate of surgical success in these eyes was 87.5% and in the eyes without inflammation was 89.4%. The difference was not significant (p>0.05)

Conclusion Subclinical inflammation, caused by antiglaucoma drugs use, was not associated with failure of filtering surgeries, in the participants of this study.

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Anterior segment optical coherence tomography imaging of OculusGen™ implants in trabeculectomy blebs

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Purpose To image blebs of phacotrabeculectomies performed with OculusGen™ implants (ProTop and MediKing Co Ltd, Taiwan) using Anterior Segment Optical Coherence Tomography (ASOCT) and compare these to mitomycin-C (MMC) augmented phacotrabeculectomies

Methods Thirty three subjects underwent phacotrabeculectomy with OculusGen implant, and 33 controls underwent phacotrabeculectomy with MMC. Blebs were imaged with ASOCT (Carl Zeiss Meditec Inc, Dublin, CA) and assessed for bleb height and area at postoperative month 1, 2 and 3

Results Mean age was similar in both groups (71.1 ±7.2 vs 69.9 ±6.8 years, p=0.67, OculusGen vs MMC). Distribution by gender and diagnosis (primary open vs closed angle glaucoma) was also similar. Mean baseline intraocular pressure (IOP) was comparable (18.4 ±4.7 vs 21.0 ±6.7mmHg, p=0.07, OculusGen vs MMC). There was no difference in mean bleb height at month 1 (1.02 ±0.34 vs 0.87 ±0.34mm, p=0.07, OculusGen vs MMC) or month 2 (0.83 ±0.26 vs 0.86 ±0.24, p=0.34), but mean bleb height at month 3 was lower in the OculusGen group (0.74 ±0.20 vs 1.00 ±0.28, p=0.00). There was no difference in mean bleb area at month 1 (2.91 ±0.54 vs 2.77 ±0.62mm², p=0.41, OculusGen vs MMC), month 2 (3.19 ±0.54 vs 3.10 ±0.73, p=0.65) or month 3 (2.97 ±0.66 vs 3.23 ±0.59, p=0.16). Mean reduction in IOP at month 3 was greater in the MMC group (2.18 ±4.93 vs 8.00 ±7.60mmHg, p=0.00, OculusGen vs MMC). At month 3, the OculusGen implants were visible in 13/33 (39.4%) subjects.

Conclusion Mean bleb height was lower in the OculusGen compared to MMC group at month 3, but mean bleb area was similar. Mean IOP reduction at month 3 was greater in the MMC group. At 3 months, OculusGen implants had not degraded in a third of eyes.

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Gene therapy mediates cone rescue and rejuvenation in the R91W mutant form of Rpe65-deficiency mice

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Purpose Given the advances of gene therapy studies to cure RPE65-derived Leber Congenital Amaurosis (LCA) (clinical trials phase I), it is of prime importance to examine how cones can be rescued in different mutant contexts. Consequently, we evaluated the effect on retinal activity and cone survival of lentivirus-mediated gene therapy in the R91W knock-in mouse model expressing the mutant Rpe65R91W gene.

Methods An HIV-1-derived lentiviral vector (LV) expressing either the GFP or the mouse Rpe65 cDNA under the control of a 0.8 kb fragment of the human Rpe65 promoter (R0.8) was produced. LV-R0.8-RPE65 or GFP was injected into 5-days-old (P5) or 1 month-old R91W mice. Functional and morphological retinal rescues were investigated at 4 months of age.

Results Increased light sensitivity was detected by ERG and pupillary light responses in animals injected with LV-R0.8-RPE65 at both P5 and 1 month compared to controls. Histological analysis showed improved expression of cone markers and cone outersegment morphology. Furthermore, the density of cones in the region of RPE65 delivery after treatment at P5 reached the wild type level. However, before injection at 1 month of age, only a fraction of the cones (40% of the number found in WT animals) in the Rpe65R91W/R91W mice expressed cone transducin, this fraction increased to 64% after treatment. Moreover, these cones appeared normal.

Conclusion We show that lentivirus-mediated Rpe65 gene transfer is very efficacious in early treatments and still efficient during the course of cone degeneration. Moreover, the treatment at 1 month shows a rejuvenation process of the diseased cones. Thus patient suffering from R91W mutation might benefit from a prolonged therapeutic window.

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Importance of electroretinogram in bull's eye maculopathy

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Purpose To describe the retinographic, electroretinographic and ultra-structural alteration in a interesting family case of bull's eye maculopathy.

Methods A 14-year-old boy, his brother a 12-year-old boy and his sister a 10-year-old girl with visual loss, underwent complete ophthalmological exams, including retinography, electroretinography (ERG) and ultrastructural study by electron microscopy of the skeletal muscle, at the Clinical Hospital of the University of São Paulo.

Results All three children presented optic nerve pallor, arteriolar thinning and bull's eye maculopathy. The scotopic responses were absent or with low amplitude contrasting with normal flicker responses. Electron microscopy study detected the curvilinear bodies typical from Neuronal Ceroid Lipofucinosi (NCL).

Conclusion The initial diagnose of those children was cone-rod dystrophy. Diagnosis of NCL was established by normal ERG flicker and findings of characteristic electron microscopic curvilinear bodies. The electrophysiologic testing are very important in the early diagnosis of NCL.

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Hunter's syndrome and buphthalmos in a girl: an unusual ophthalmic association

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Purpose To report an unusual ophthalmic presentation of a case of Hunter's syndrome/ MPS II.

Methods A sixteen-year-old girl presented to us with total loss of vision and forward protrusion OU since early childhood. Detailed examination, including slit lamp biomicroscopy, Intra ocular pressure (IOP) and funduscopy was carried out. Thorough systemic evaluation including Computed Tomography (CT), metabolic and genetic analysis was undertaken in collaboration with internists.

Results Characteristic facies, detection of glycosaminoglycan (GAG) variants in urine (chondroitin sulfate B and heparin sulfate) and iduronate-2-sulphatase activity in fibroblasts/leucocytes confirmed the diagnosis of MPS II. Child had severe photophobia but with no perception of light OU. OU buphthalmos with Haab's striae was noted, making a clear view of the fundus difficult. IOP OU was elevated, and 90D slit lamp biomicroscopy revealed a total glaucomatous optic atrophy in both eyes. On CT there was thickening and edema of preseptal and periorbital soft tissue with marked thinning of the optic nerves with prominent perineural CSF sleeves, indicative of marked optic atrophy.

Conclusion Glaucoma is a known association of Hurler's, Scheie's and Maroteaux-Lamy syndromes but not Hunter's. In fact, there is only one report of suspected angle closure glaucoma in MPS II. Buphthalmos is not a likely presentation as the sclera in these patients is known to be thickened due to deposition of GAG. To the best of our knowledge, this is the first case report of buphthalmos in association with MPS II. The importance of a meticulous examination in this subset of patients cannot be overemphasised. An appropriate and timely intervention may result in a better quality of life for them.

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LOC387715/ARMS2 studies – gene sequencing as a procedure of choice

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Purpose To search for important mutations in LOC387715/ARMS2 gene.

Methods 80 patients with choroidal neovascularization subsequent to age-related macular degeneration undergoing anti-VEGF treatment were screened for LOC387715/ARMS2 mutations. PCR followed by gene sequencing was performed. If polymorphism A69S coexisted with R38STOP gene cloning with pGEM-T vector was done.

Results R38STOP mutation was found in 5 patients, A69S in 59 patients, in 3 cases R38STOP coexisted with A69S on the other allele. Due to substantial shortening of the R38STOP translation product, the protein is probably not effective. In patients with A69S polymorphism coexisting with R38STOP there is only A69S effective allele – so patients should be treated as A69S homozygous.

Conclusion There is increasing number of studies with A69S variant of ARMS2 but only gene sequencing provide reliable data in these cases. If R38STOP is taken into account, A69S is even more important AMD risk factor. Gene sequencing is advisable in studies with LOC387715/ARMS2.

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Relationship between IOP and age in the Salzburg-Moorfields-Collaborative Glaucoma Study

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Purpose To investigate the distribution of intraocular pressure in a population based survey in the county of Salzburg.

Methods The SMCGS is a single-center, prospective cohort study embedded into a government supported glaucoma blindness prevention program in Salzburg county, Austria. A complete ophthalmological examination including biomicroscopy of the optic nerve head, visual field testing, measurements of intraocular pressure, central corneal thickness and laser scanning topography of the optic disc was performed on 3650 subjects at the beginning of the study as well as at the five year follow-up.

Results The average age is 60.3 years (min: 40.04, max: 93.9 years), IOP is 15.0 mmHg (min: 7, max: 32 mmHg) and the correlation coefficient between age and intraocular pressure is $r = 0.005$, $p = 0.75$ (95% CI -0.03 and 0.026). Also in the subgroups (people with normal findings, people with increased cup to disk ratio (C/D>0.45) and people with ocular hypertension (IOP>21mmHg) no significant correlation between age and intraocular was found.

Conclusion In the past, numerous studies showed a correlation between age and intraocular pressure or at least reported a vague relationship between the two variables. Of all large scale studies only the blue mountain eye study and the Beijing eye study did not find a positive correlation between age and intraocular pressure (before correction for arterial pressure). In the population of Salzburg, age is an independent risk factor for glaucoma, independent of IOP and without correction of possible confounders.

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Major eye diseases and risk factors associated with systemic hypertension in an adult chinese population: the Beijing Eye Study

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Purpose To assess the relationship of hypertension with major eye diseases and other ocular parameters.

Methods The Beijing Eye Study is a population-based study. Examination at baseline in 2001; follow-up examination in 2006; 3222 subjects had blood pressure measurements. All participants underwent a thorough ophthalmic examination and blood pressure measurement. Hypertension was defined as a systolic blood pressure ≥ 140 mm Hg and/or a diastolic blood pressure ≥ 90 mm Hg, and/or self-reported current treatment for hypertension with antihypertensive medication.

Results Mean age of participants in the present study was 60.4 ± 10.0 years. Hypertension was present in 1500 (46.6%) of the 3222 subjects who had their blood pressure measured. In multiple regression analysis, hypertension was associated with higher intraocular pressure ($P = 0.005$), arterio-venous nicking ($P = 0.009$), retinal vein occlusions ($P = 0.02$), and diabetic retinopathy ($P = 0.02$). Hypertension was not significantly associated with the prevalence of open-angle glaucoma ($P = 0.19$) or angle-closure glaucoma ($P = 0.15$), age-related macular degeneration ($P = 0.73$), nuclear cataract ($P = 0.88$), posterior subcapsular cataract ($P = 0.30$), cortical cataract ($P = 0.10$), or area of alpha zone ($P = 0.05$) or beta zone of parapapillary atrophy ($P = 0.95$).

Conclusion In Chinese persons, while controlling for other systemic parameters, hypertension was associated with increased intraocular pressure, retinal microvascular abnormalities, and prevalence of retinal vein occlusion and diabetic retinopathy. Hypertension was not associated significantly with age-related macular degeneration, age-related cataract, or glaucoma

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Screening history of those with severe visual impairment due to diabetic retinopathy

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Purpose To examine screening activity prior to blind registration due to diabetic retinopathy (DR) in NI. To identify any grading misclassifications and risk factors for blindness in those screened.

Methods Details on all registered blind due to diabetic retinopathy were obtained from the statutory register (SOSCARE). Demographic data (surname, date of birth and sex) were used in any combination to ascertain previous screening within the evolving NI Diabetic Retinopathy Screening Programme. Screening history and clinical chart review formulated the history prior to registration.

Results The prevalence of diabetic blindness amongst the diabetic population was 0.34%. (171 people) 56% were female and the average age was 65.6 (SD 15.6). Diabetic maculopathy was cited as the commonest cause (83%) with proliferative disease in 17%. 84% had never been screened and were under the care of an eye clinic. 27 people (16%) had been screened within the previous 4 years. 22 of these had been discharged from eye clinics post registration. 5 had never attended an eye clinic. 4 of these showed maculopathy on screening and were referred to an ophthalmologist. One person aged 31, diabetic for 20 years, was screened 2 years prior to registration due to PDR. The screening result was given as no retinopathy in either eye. The digital images were reassessed and no change in grade made. Detailed scrutiny of clinical notes revealed a recent history of severe anorexia with insulin used to control weight.

Conclusion Timely referral from screening is required for optimal management, but it must be recognised that vision cannot be maintained in all cases. Risk factor stratification for earlier screening in exceptional circumstances may be wise.

Commercial interest

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Nitric oxide synthesis is related to astroglial cell cycle in the mouse retina via p53 gene regulation

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Purpose To analyze the influence of p53 gene in retinal astrocytes morphology and function.

Methods Adult mice of the strain C57BL/6 were distributed into two groups: 1) wild type TP53, as the controls (cp53; n=20) and 2) mice with an extra copy of p53 (sp53; n=22). Both eyeballs were enucleated, after death by ether narcosis, to obtain the retinas. Right retinas were frozen (-85°C) until processing to assess nitric oxide (NO) synthesis. Left retinas were obtained and processed to perform immunohistochemical localization of the glial fibrillary acidic protein (GFAP). Data are expressed as the mean \pm SD. Statistical analysis was carried out using Student's t-test. Confidence interval was taken at 95%.

Results The NO levels were significantly higher in the sp53 retinas than in the cp53 ones (145 ± 15 vs 111 ± 10 μ M; $p < 0.05$). Strong GFAP expression was detected and mapped by fluorescence microscopy in the whole retinas. GFAP+ stellate cells were located throughout the peripheral, equatorial and central retina. Astroglial density was significantly higher in the sp53 retinas as compared to the cp53 group (72 ± 8 vs 49 ± 6 cells/retinal area; $p < 0.001$).

Conclusion All data suggest a complex set of p53 actions: 1) to regulate NO production (probably recruiting a latent retinal astrocytic population), 2) to regulate the sensitivity toward NO in the retina by protecting astrocytes from oxidative attack. Increasing p53 activity may be an outstanding therapeutic strategy for certain retinal diseases.

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Symmetry of retinopathy in the NI diabetic retinopathy screening programme NIDRSP

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Purpose To predict the probable level of retinopathy in an ungradeable image from the retinopathy status in the gradeable fellow eye

Methods Retinopathy is graded within NIDRSP on a well established grading scale (0-6) based on the ETDRS scale. The retinopathy status (screening grades) was taken from a cohort of 5000 consecutive patients. Inter eye correlations in grading outcome were examined

Results A high degree of symmetry in retinopathy grades was found. At the lower levels of grade the right eye tended to be slightly worse than the left. If a retinopathy grade of 0 (none) to 2 (definite early retinopathy) was found in the right eye. If no retinopathy was found in 1 eye (right or left), the probability of having referable retinopathy in the fellow eye was found to be zero. If moderate non referable retinopathy was found in the right eye, the probability of having referable retinopathy in the left was 8%. If moderate non referable retinopathy was found in the left eye, the probability of having referable retinopathy in the right was 6%. If referable retinopathy was found in the right eye, referable retinopathy was present in the left in 60%. If referable retinopathy was found in the left eye, referable retinopathy was found in the right eye in 46%.

Conclusion At more advanced levels of retinopathy the probability of having sight threatening retinopathy is higher for the left eye than for the right. This should be considered when referring patients in whom the retinal status of one eye is masked by eg media opacities.

Commercial interest

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency and proliferative diabetic retinopathy in a Sardinian male population, Italy

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Purpose Purpose: The reported incidence of G6PD deficiency in Sardinia, Italy, ranges from 10% to 15%. Hemizygous males have totally deficient erythrocytes. Evidence indicates that patients with G6PD deficiency are protected against ischemic heart and cerebrovascular disease, retinal vein occlusion, and nonarteritic anterior ischemic optic neuropathy. The purpose of this study was to assess the incidence of G6PD deficiency in Sardinian men with proliferative diabetic retinopathy (PDR) and ascertain whether G6PD deficiency may have a protective effect against this vascular disorder.

Methods Methods: G6PD blood levels were measured in 524 men with PDR. 1262 non-diabetic men undergoing cataract surgery served as controls. The Z test was used to assess differences in G6PD deficiency prevalence rates between groups. The odds ratio (OR) was used to evaluate the association between G6PD deficiency and PDR.

Results Results: G6PD deficiency was found in 30 (5.7%) out of 524 patients with PDR and in 107 (8.5%) out of 1262 controls. Differences between PDR patients and controls were statistically significant ($Z = 1.99$, $P < 0.046$). G6PD deficiency showed a statistically significant protective effect against PDR (OR: 0.66; 95% Confidence Interval: 0.43-0.99).

Conclusion Conclusions: The prevalence of G6PD deficiency in Sardinian men with PDR was lower than expected. Results suggest that G6PD deficient men have a lower risk of developing PDR.

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Phenotype-genotype correlations of TGFBI in Asian patients

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Purpose Phenotypic-genotype correlations of mutations in Tissue Growth factor beta-inducible (TGFBI) responsive gene in Asian patients with Bowman's membrane and stromal corneal dystrophies.

Methods Twenty families with Bowman's membrane and stromal corneal dystrophies presenting to a tertiary referral centre underwent visual acuity testing, and ocular examination with slit lamp biomicroscopy. Blood was collected for genetic analysis and the TGFBI gene was screened for possible mutations.

Results There were 10 males and 10 females in this series, with 10 Chinese, 4 Malays, 4 Indians and 2 Indonesians. The mean age was 48.9 years (range 9 - 72). Five (out of 9) patients had the R555W mutation, while the other 4 had the R124H mutation. Of the 9 patients with lattice dystrophy, 2 had the H626R mutation while 5 had the R124C. One novel mutation Ala620Asp in a patient with lattice dystrophy was identified. Other mutations detected include R124L, R555Q and H572R. There was marked phenotypic variation amongst different TGFBI mutations.

Conclusion The most common mutation amongst our patients was R555W and R124C. We describe the clinical features of a previously unreported TGFBI mutation. There was marked phenotypic variability in TGFBI mutations in Asian patients.

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Genetic and phenotypic analyses of retinitis pigmentosa in northern Tunisia

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Purpose To describe the different types of retinitis pigmentosa (RP) in Northern Tunisia and to determine the genetic form.

Methods A prospective non-comparative study was conducted. Different forms of RP disease were diagnosed in 99 patients from 56 families living in northern Tunisia. One hundred eighty individuals had ophthalmic examination including best corrected visual acuity, slit lamp biomicroscopy, fundus photography, and in some cases fluorescein angiography, OCT and electroretinogram (ERG). Clinical features, mode of presentation and mode of inheritance were noted. Blood samples were collected for genetic analyses. Gene RPE65 was screened in all affected cases.

Results Half of families presented first degree consanguinity. Sixty eight percent of cases were inherited by autosomal recessive transmission. Sporadic cases were estimated to 32%. Patients mean age at initial examination was 36 years (range: 2 to 70 years). Disease onset mean age was 15 years. Severe progression of disease with constriction of visual field, low vision and extended retinal atrophy was seen in 52% of patients. Among the 99 cases of RP, there were 80 common forms, 9 Usher's syndrome, 4 congenital amaurosis of Leber, 3 progressive cone-rod degeneration, 1 paravenous form and 1 unilateral form. Mutation R19W of RPE65 gene was detected in two families.

Conclusion RP is a group of clinical and genetic heterogeneous diseases that can lead to blindness. RP in northern Tunisian population presents some particularities. The recessive severe form is largely predominant probably due to the elevated rate of consanguinity in Tunisia.

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Central corneal thickness and its association with ocular and general parameters in Indians. Central India Eye and Medical Study

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Purpose To evaluate the distribution of central corneal thickness and its associations in the adult Indian population

Methods The Central India Eye and Medical Study is a population-based study performed in a rural region close to Nagpur in Central India and included 4711 subjects (aged 30+ years) out of 5885 eligible subjects. This study was focused on central corneal thickness (CCT) as measured by sonography and its associations. Intraocular pressure was measured by applanation tonometry.

Results CCT measurement data were available for 9370 (99.4%) eyes. Mean CCT was 514 ± 33 μ m (median: 517 μ m; range: 290-696 μ m). In multiple regression analysis, CCT was significantly associated with male gender ($P < 0.001$), higher body mass index ($P = 0.028$), lower age ($P < 0.001$), lower corneal refractive power ($P < 0.001$), and higher anterior chamber depth ($P = 0.025$). CCT was not significantly associated with axial length ($P = 0.39$), lens thickness ($P = 0.65$), refractive error ($P = 0.12$) and cylindrical refractive error ($P = 0.75$). Intraocular pressure readings increased significantly ($P < 0.001$) with higher CCT and higher corneal refractive power

Conclusion Indians from rural Central India have markedly thinner corneas than Caucasians or Chinese. As in other populations, CCT is higher in males. It decreased with higher age and lower body mass index. Intraocular pressure readings were falsely high in eyes with thick corneas and steep corneas. Besides corneal thickness, the anterior corneal curvature has to be taken into account in applanation tonometry. It may hold true particularly after corneal refractive surgery.

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Differential association of cataract sub-types with obesity and FTO polymorphisms

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Purpose Investigating a possible link between genetic variants associated with obesity and cataract may validate the causal link between obesity and cataract suggested by epidemiological studies. This study assessed the associations of obesity and genetic variants in the FTO locus, a human susceptibility locus for obesity, with cataract.

Methods This was a population-based cross-sectional study on adults aged 40-80 years. Cataract was assessed by standardized slit-lamp examination according to the Lens Opacity Classification System III (LOCS III). Obesity was defined by BMI ≥ 30 kg/m². The SNP rs9939609 at the FTO locus was selected for analysis. Additive and recessive models were constructed for the association between FTO polymorphisms and cataract.

Results 3069 subjects were included, of which 1476 (48.1%) had any cataract, 927 (30.2%) had nuclear cataract, 1012 (33.0%) had cortical cataract and 308 (10.0%) had PSC cataract. After multivariable adjustment, obesity was significantly associated with cortical (OR 1.34 (95% CI 1.04-1.73)) and PSC (OR 1.53 (1.07-2.18)) cataracts, but not nuclear cataract. In the additive model, each copy of the minor allele of rs9939609 was associated with nuclear cataract (OR 1.33 (1.11-1.58)). This association persisted in multivariate analyses controlling additionally for BMI, diabetes, hypertension and smoking (OR 1.30 (1.08-1.55)). There was no association with cortical or PSC cataract. In the recessive model, rs9939609 was associated with nuclear but not cortical or PSC cataract.

Conclusion Obesity is associated with cortical and PSC cataract while the SNP rs9939609 in the FTO gene is associated with nuclear cataract. The contrasting associations may reflect differences in the pathophysiology of cataract subtypes.

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Severe ocular trauma cases: types and incidence

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Purpose Presentation of severe traumatic ocular injuries during the year 2008 at Ophthalmology Department of Hippokraton General Hospital in Thessaloniki

Methods The study included patients who came at our clinic with severe ocular trauma and needed admission for observation and/or surgical treatment (total number 26)

Results Included cases were: I) 9 (34,6%) cases of blunt trauma (4 with hemophthalmia and 5 with hyphaema), only 2 needed surgical evacuation. II) 6 (23,1%) corneal penetrating traumas with intraocular foreign body, all of which surgically treated, with mild visual impairment in 3 cases. III) 8 (30,7%) cases of corneal penetrating trauma without intraocular foreign body, 6 surgically treated, with good visual outcome in 6 cases, IV) 3 (11,6%) cases of scleral laceration without intraocular foreign body, all surgically treated, 1 of them with severe visual impairment

Conclusion Severe traumatic ocular injuries are frequent at Ophthalmology Department of Hippokraton General Hospital (most frequent blunt trauma followed by corneal penetrating trauma) and with timely treatment the majority of cases had good visual outcome.

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A novel mitochondrial ND5 gene mutation m.13042G>A in Leber hereditary optic neuropathy

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Purpose To report clinical and genetic characterization of two related patients with Leber hereditary optic neuropathy (LHON).

Methods A 20-year old man was referred in September 2004 for acute, painless and severe visual loss in his left eye, and then three weeks later in the other. Visual acuity (VA) on presentation was 0.2 in his right eye and HM in his left eye. His 11-year old cousin was referred in December 2006 for acute and painless visual loss on both eyes with VA of CF in his right eye and 0.3 on his left eye. Fundus examination in both patients revealed engorged optic disc with telangiectatic and tortuous vessels with no leakage on fluorescein angiography. MRI of brain was normal in both patients. There were loss of N95 wave in PERG and abnormal VEP suggesting retinal ganglion cell loss and optic nerve disease. In first patient, VA decreased to HM on both eyes in few months and in 4,5 year follow up didn't improve. In second patient, VA decreased to 0.001 on right eye and 0.04 on left eye in 2,5 year follow up.

Results Genetic tests of the mitochondrial (MT) DNA for three most common mutations (m.11778 G> A, m.3460 G>A, m.14484 T>C) responsible for LHON were negative in both patients. Use of Mito-Chip 2 didn't reveal any mutations, whilst sequencing the entire MT-genome in the first patient revealed mutation m.13042G>A (homoplasmy) in gene MT-ND5; for second patient sequencing for this mutation is in process.

Conclusion This mutation has not been previously described in association with LHON and was not mentioned in mtDB or mtSNP (single nucleotide polymorphism) database (DB). The mutation is likely to be causative of the disease since no other mutations were found in MT-genome in a patient with typical clinical presentation of LHON.

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Ocular features and management in the mucopolysaccharidoses

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Purpose To evaluate the ocular findings and the visual outcome in a group of patients with mucopolysaccharidoses (MPS) followed and treated with new therapeutic options.

Methods 18 patients with MPS (9 males and 9 females) were examined: 3 cases with MPS type I, 4 with MPS type II, 7 with MPS type III, 1 with MPS type IV and 3 with MPS type VI. The patients underwent an ophthalmological assessment with: visual acuity (with Teller Acuity Cards, Lea Symbols or letter chart, depending on patient age or degree of cooperation), slit-lamp examination, fundus ophthalmoscopy, intraocular pressure, refractometry, electroretinography and eye echography.

Results The mean follow-up was 48 months (ranged from 3 to 190 months). The mean age at first ophthalmologic evaluation was 6,9 years (ranged from 1 to 20 years). 8 cases presented progressive corneal clouding, 5 cases retinal pigmentary degeneration, and 1 papilledema. Visual acuity worsened severely in 3 cases with MPS type I: 1 case from 10/10 to 5/10, 1 case from 5/10 to 1/10; in another case visual acuity decreased to 1/20 and underwent to corneal transplantation with good visual outcome (RE 5/10 and LE 7/10). The other cases showed slightly reduction of visual function. 3 patients had ocular hypertension and were treated with hypotensive eye drops. 8 patients underwent enzyme replacement therapy.

Conclusion The mucopolysaccharidoses (MPS) are rare systemic disorders caused by accumulation of glycosaminoglycans. Ophthalmological manifestations are frequent in MPS, particularly in MPS I, II and VI, characterized by corneal clouding, retinal dystrophy and blindness. Regular ophthalmic monitoring to determine disease progression and management of complications are necessary as a part of multidisciplinary approach.

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Cytochrome C oxidase expression in retinal rod outer segment disks

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Purpose The rod outer segment (ROS) plays a pivotal role in the phototransduction cascade, a process which requires a high level of energy consumption. In rods ATP production is confined to the mitochondria in the inner part, and the distances between sites of energy production and expenditure do not seem adequate to supply phototransduction. The goal of this study was to test the hypothesis that Cytochrome c oxidase (Cox) is located and catalytically active in ROS disk membranes.

Methods Osmotically intact disks were isolated from 20 bovine retinal ROS obtained by sucrose gradient centrifugation in the presence of protease inhibitor cocktail and Ampicillin under dim red light at 4°C. Cox expression was studied by confocal laser scanning microscopy and semiquantitative Western-immunoblotting, with a specific antibody. Cox activity in disks was investigated spectrophotometrically.

Results Results from immunohistochemical confocal laser scanning microscopy, Western blot, and biochemical experiments suggest that Cox is uniformly distributed onto disks and therein catalytically active to an extent comparable to that of isolated mitochondria.

Conclusion The expression and activity of Cox in bovine ROS disks is suggestive of an aerobic metabolism in ROS. This study opens new scenarios on the energy production in rods and on the possible role of its deficiency in some important retinal diseases which have not been clarified yet.

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In vitro transcorneal and transscleral diffusion of radiolabeled compounds in human and rabbit cornea and in human, monkey, dog, and rabbit sclera

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Purpose To determine the in vitro diffusion of selected compounds across the cornea or sclera of humans, monkeys, dogs, and rabbits.

Methods Human and NZW rabbit corneas were obtained and the epithelium was removed from one cornea of each pair. Corneas were mounted in chambers with 3H-water or 3H-dexamethasone on the epithelial side and BSS solution on the endothelial side. Serial aliquots were taken from each chamber and assayed by LSC. Scleral sections from human, NZW rabbit, dog, or monkey eyes were mounted in perfusion chambers. Adjacent sclera was used for H&E histology. 3H-water, 3H-dexamethasone, or 70kD-14C-dextran were added to the episcleral surface while perfusing BSS across the choroidal side. Serial aliquots were collected up to 5 hrs and assayed by LSC. Scleral permeability (ktrans) was calculated. H&E slides were used to determine scleral thickness.

Results 3H-water diffused through cornea faster than 3H-dexamethasone, especially with denuded epithelium. Scleral thickness and molecular weight were determinant of diffusion in sclera. Monkey sclera was thinnest, followed by dog, rabbit, and human. ktrans for 70kD-14C-Dextran and 3H-dexamethasone were greatest in monkey, followed by dog, rabbit, and human. ktrans values for 3H-water were similar in all species, and greater than values for 14C-dextran and 3H-dexamethasone.

Conclusion These studies demonstrate permeability of cornea and sclera in human and animal models with compounds of varied molecular weights representative of drugs being developed for treatment of ocular diseases. The results of this study indicate that these techniques are valuable as screening tools in the development of ocular drugs.

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Vasopressin receptors in ocular tissues and their impact on ocular hydrodynamics

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Purpose To investigate the effect of intravenous applied vasopressin on aqueous flow and to localize involved receptor types in ocular tissues.

Methods In anesthetized rabbits mean arterial pressure (MAP), intraocular pressure (IOP) and orbital venous pressure (OVP) were measured by direct cannulation of the central ear artery, the vitreous, and the orbital venous sinus, respectively. Laser Doppler flowmetry was used to record CilBF continuously. Aqueous flow (AF) was measured simultaneously by fluorophotometry. After baseline measurements arginine-vasopressin (AVP) was applied intravenously (infusion rate: 0.08 ng/kg/min). Immunostaining (SABC-method, immunofluorescence) was performed to localize potential vasopressin receptors in ocular tissues.

Results AVP caused a considerable increase of MAP (+6.90±1.21%; p < 0.001), a significant decrease of IOP (-9.56±2.35%; p = 0.003), a highly significant reduction of AF (-21.34±4.08%; p < 0.001) and no significant change of CilBF (-2.37±3.38%; n.s.). Immunofluorescent stainings show a characteristic pattern of V1- and V2-receptor distribution. Both, V1 and V2 receptors are detectable in choroidal, ciliary and iridal vessels. Moreover, they are localized in the ciliary epithelium, whereas the labeling is more intensive in the non-pigmented epithelium than in the pigmented epithelium.

Conclusion Although the applied dose of AVP did not changed CilBF considerably, a highly significant reduction of AF was observed. This suggests that the reduction of AF is predominantly caused by affecting the secretory mechanisms in the ciliary epithelium. The localization of vasopressin receptors in ciliary epithelium supports this assumption.

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Consequences of dietary omega-3 polyunsaturated fatty acid deficiency on retinal function and intraocular pressure in the rat

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Purpose Omega-3 polyunsaturated fatty acids (ω3) are key components in nervous structures but their dietary intakes in the overall population are often below nutritional requirements. A chronic deficiency in ω3 is recognized to be associated with functional impairment of the retina. At the opposite, ω3 supplementation is associated with a reduced risk for AMD. The consequences of ω3 deficiency on other eye structures than the retina, such as ciliary bodies, are scarce. The purpose of our study was to compare the response of the retina and ciliary bodies to dietary ω3 deficiency in terms of fatty acid profile and eye functionality.

Methods Two successive generations of Lewis rats (G1 and G2) were obtained under either a standard or ω3-deficient diet. Intraocular pressure (IOP) was measured by rebound tonometry throughout the experiment. Retinal functionality was assessed by scotopic electroretinography (ERG). Gas chromatography was used to determine the fatty acid profile of the ciliary bodies and retina.

Results A 2-fold fall in DHA content of the retina was observed in ω3-deficient G1 animals. This decrease was accentuated in G2 (-66%) and counterbalanced by an increase in DPAω6 in the retina. The b-wave amplitude of the ERG was decreased by 50% at 9mcds/m² in ω3-deficient rats. In ciliary body DHA was reduced by 80% in ω3-deficient in G1 animals but not in G2. Meanwhile, animals from the ω3-deficient diet had increased IOP (18 vs 12mmHg, p<0.0001).

Conclusion The crucial role of ω3 in retinal function was confirmed. The most relevant finding from our study is the rise in IOP, the major risk factor for glaucoma, which was observed in animals reared under dietary deficiency in ω3.

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Butaprost, a prostaglandin EP2 receptor agonist, counteracts the negative effect of serum deprivation to retinal ganglion cells

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Purpose To investigate the neuroprotective role of butaprost an EP2 receptor agonist.

Methods Cultures of transformed retinal ganglion cells (RGC-5 cells) were maintained in normal medium or medium that lacked serum for 48 hours. During this period butaprost was added in some cases to the medium. The cultures were then subjected to various methodologies (MTT-assay, WST-assay, APOPercentage analysis, TUNEL, DHE staining, LDH assay, flow cytometric analysis and immunohistochemistry) so as to characterize their nature of survival. The level of cAMP was also measured in some cases by ELISA.

Results Functional EP2 receptors in RGC-5 cells were demonstrated by immunohistochemistry, western blot and by the generation of cAMP caused by the presence of butaprost. Serum deprivation resulted in RGC-5 cells dying by apoptosis, indicated by APOPercentage⁺, TUNEL and flow cytometric assays. Butaprost (1 or 10 μM) added 30 minutes after the onset of serum deprivation significantly reduced cell death caused by serum deprivation.

Conclusion The EP2 agonist butaprost significantly attenuates apoptosis caused by serum deprivation. This data supports the view that EP2 agonists might have a role to play in the treatment of glaucoma given that it is known to lower IOP significantly.

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Ocular and plasma bioavailability of different memantine formulations after periocular administration

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Purpose To determine the memantine vitreous and plasma levels after the periocular injection of different types of memantine formulations, saline solution (SS), methylcellulose 0.5% (MC 0.5%) and polyvinylpyrrolidone 1% solution (PVP 1%); by posterior subtenon route.

Methods In vivo experiments were done with albino New Zealand rabbits. Forty five rabbits were used and divided into 3 groups, one for each of the essayed formulations. 4,5 mg of memantine in 0,3 ml of formulation were administrated per animal by periocular injection into the posterior subtenon space, on the right eye of each animal. Plasma (2mL) and vitreous humor samples were obtained at 10, 60, 120, 240, and 1440 minutes post injection. A method with n-hexane liquid-liquid extraction and FMOC derivatization was used for the quantitative determination of memantine in the biological samples, using HPLC with fluorescence detection.

Results Just 10 minutes post injection, detectable levels of memantine in plasma and vitreous humor were obtained on each of the 3 groups. Several concentrations of memantine were detected in vitreous from 10 to 1440 minutes when PVP formulation was administered. However, 24 hours post injection, no Memantine levels were detected on treated eyes with SS or MC 0,5% solution. According to plasma similar concentrations of memantine have been obtained for the different types of formulations.

Conclusion After periocular administration, several memantine levels were detected in the vitreous chamber. In our opinion, these concentrations can be higher than after systemic and topic administration. The use of formulations as PVP, which has a higher permanence on the injected area, allows a sustained delivery of the drug.

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N-Acetyl cysteine (NAC) attenuates neuronal cell death in culture not solely by up regulating glutathione (GSH).

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Purpose To show that NAC attenuates GB (glutamate/buthionine sulfoximine)-induced death to retinal ganglion cells (RGC-5 cells) in culture by a mechanism that does not solely involve GSH.

Methods Transformed retinal ganglion (RGC-5) cells grown in culture were exposed to an insult of GB for defined periods. NAC at different concentrations or vehicle was added to the cultures over 24 hours. Reactive oxygen species (ROS) generation was measured using a dye 20,70-dihydroethidium. Cell death was measured by use of an MTT and resazurin-assays and evidence for apoptosis was obtained with an APOPercentage⁺ procedure. Glutathione (GSH), catalase (CAT), glutathione-S-transferase (GST) and superoxide dismutase (SOD) were also determined using a different fluorescent procedure.

Results NAC dose-dependently attenuated GB-induced generation of ROS and death by a form of apoptosis to RGC-5 cells in culture. NAC also independently stimulated the production of not only GSH but also GST, CAT and SOD. Moreover, NAC attenuated GB-induced loss of intracellular GSH, CAT and GST.

Conclusion NAC significantly blunts oxidative-induced death to RGC-5 cells induced by GB. Evidence is also provided to show that NAC increases intracellular GSH, GST, CAT and SOD levels. The neuroprotective action of NAC is therefore complex and is not solely caused by an action on GSH.

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Choroidal blood flow regulation during a latanoprost-induced decrease in intraocular pressure

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Purpose Blood flow regulation in the human choroid appears to be complex. We have recently shown that choroidal blood flow is better regulated during exercise-induced changes in choroidal blood flow than during a suction-cup-induced increase in intraocular pressure (IOP). In the present study we hypothesized that choroidal blood flow regulation may be altered during a latanoprost-induced decrease in IOP.

Methods In this prospective, balanced, placebo-controlled parallel group study, 18 healthy male volunteers were included. Choroidal blood flow regulation was assessed using laser Doppler flowmetry during an artificial increase in intraocular pressure using a suction cup and during a 6 minutes squatting period. Results were compared before drug administration and after 14 days of topical instillation of either latanoprost or placebo.

Results Neither systemic blood pressure nor choroidal blood flow at baseline was altered by latanoprost. As expected, latanoprost did, however, reduce IOP. The regulation of choroidal blood flow was altered by latanoprost with a wider range of regulation compared to placebo treatment (p < 0.05).

Conclusion The present data indicate that administration of latanoprost improves choroidal blood flow regulation. Since latanoprost did not alter baseline choroidal blood flow this effect may be related to the reduction in IOP.

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Urocortin2 relaxes the iris sphincter muscle

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Purpose Urocortin 2 (Ucn2) is an endogenous peptide of the corticotropin releasing factor family which presents many physiological effects at different levels in the organism. At the ocular level, Ucn2 is expressed in the retinal pigmented epithelium and has a protective effect in retinal degeneration pos-ischemia. Also in the eye, Ucn2 has a vasodilator effect in the retinal circulation, specifically, in retinal resistance arterioles. This effect is diminished in diabetic mice. However, it has not been described yet the role of Ucn 2 in the neurohumoral regulation of the anterior segment of the eye. Our purpose is to characterize the effect of Ucn2 and its sub-cellular pathways on iris sphincter muscle contraction.

Methods We tested the effect of increasing concentrations of Ucn2 (10-10-10-6 M) on carbachol-precontracted (10-6 M) rabbit iris sphincter muscles (n = 9). The effect of Ucn2 was also tested in the presence of: (i) N ω -nitro-L-Arginine (L-NA; 10-5 M; n = 13) and (ii) indomethacin (10-5 M; n = 7).

Results Ucn2 promoted a concentration-dependent decrease on active tension of the precontracted iris sphincter muscles, with maximal effect at 10-6M (12,9 \pm 4,1%). This effect was blunted with indomethacin (2,1 \pm 4,4%) and with L-NA (2,17 \pm 5,79%).

Conclusion Ucn2 promotes the relaxation of iris sphincter muscle. This effect is mediated by prostaglandins and it is also dependent on nitric oxide production. As observed Ucn2 is a new neurohumoral factor that modulates the relaxation of iris sphincter muscle.

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Intravitreal L-arginine injection reverses the retinal arteriolar vasoconstriction that occurs after experimental acute branch retinal vein occlusion

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Purpose To investigate the effect of L-Arginine on the retinal arteriolar diameter following acute branch retinal vein occlusion (BRVO) in minipigs.

Methods Under general anesthesia, 10 eyes of 10 minipigs were evaluated. Two hours after BRVO, an intravitreal juxta-arteriolar microinjection of 30 μ l L-Arginine 1 mM (pH=7.4) was performed in 7 eyes. Three eyes received a microinjection of 30 μ l of the solvent (pH=7.4) that was used to prepare the solution of L-Arginine and served as controls. Retinal arteriolar diameter changes were measured using a Retinal Vessel Analyzer.

Results Overall (n=10), two hours after BRVO there was a 10,5 \pm 1,9 % decrease in the retinal arteriolar diameter in the affected territories compared to baseline (p<0.05). An increase of 16,0 \pm 3,0 % (p< 0.01) and 21,0 \pm 7,0 % (p<0.05) of the arteriolar diameter was evidenced 10 and 15 minutes respectively after L-Arginine injection (n = 7) compared to the diameter prior to L-Arginine injection. Thereafter, the vasodilatory effect of L-Arginine started to decrease but persisted and remained significant at the end of the study period (5,0 \pm 1,5 % at 30 min, p<0.05). Microinjection of the solvent that was used to prepare the solution of L-Arginine (n=3) did not produce any significant effect on the retinal arterioles, which remained constricted at all time-points (p>0.1).

Conclusion We found a significant vasodilation after intravitreal juxta-arteriolar L-Arginine microinjection in eyes with experimental BRVO. L-Arginine microinjection can be a beneficial therapeutic option in acute BRVO, reversing the arteriolar vasoconstriction by stimulating NO production.

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Choroidal blood flow response to hyperoxia and hypercapnia in patients with obstructive sleep apnea syndrome

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Purpose Obstructive sleep apnea syndrome (OSA) is associated with ischemic and glaucomatous optic neuropathy. The physiopathology of these diseases includes an abnormal regulation in the optic nerve head vasculature. The aim of our study was to characterize the choroidal vascular reactivity to hyperoxia and 8% CO₂ breathing in OSA patients, as compared with matched healthy control subjects.

Methods Eighteen newly diagnosed OSA patients were included in this prospective study. Control subjects were matched with OSA patients for body mass index (BMI), gender and age. At the screening visit, each subject underwent a general exam, cardiovascular, neurologic and ophthalmological examinations, and underwent complete overnight polysomnography. The LDF instrument used in this study to measure subfoveal choroidal blood flow (ChBF) measured the following parameters: ChBVel (kHz); volume, ChBVol (in arbitrary units, AU) and the relative flow, ChBF = ChBVel / ChBVol (in AU). Vascular choroidal reactivity was tested during hyperoxia and hypercapnia (8% CO₂) inhalation.

Results OSA patients exhibited a similar choroidal reactivity during hyperoxia (stability of choroidal blood flow) and hypercapnia (1.5% increase in choroidal blood flow per 1 mmHg PtCO₂) than controls.

Conclusion This prospective comparative study explored for the first time the choroidal blood flow of OSA patients and results showed that subfoveal ChBF reactivity to gas in OSA patients is similar to healthy control subjects. These preliminary results suggest that choroidal vasculature is protected against the imbalance between the vasodilation (NO) and vasoconstriction (endothelin) system.

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The effect of betablockers on ocular hemodynamics in anesthetized rabbits

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Purpose To compare the effects of different betablockers on ocular hemodynamics in anesthetized New Zealand White rabbits.

Methods Mean arterial pressure (MAP), intraocular pressure (IOP) and orbital venous pressure (OVP) were measured by direct cannulation of the central ear artery, the vitreous and the orbital venous sinus, respectively. Laser Doppler flowmetry was used to measure choroidal and ciliary blood flow. The performed measurements were made continuously at baseline and after drug application.

Results Metoprolol showed a significant reduction of the choroideal blood flow (-13,6 \pm 1,4%, p = < 0,05) which was not observed in the ciliary body. Atenolol had a similar effect on the choroid with a significant reduction (-7,3 \pm 1,0%, p< 0,05) and no significant change in the ciliary body. The intravenous administration of Nebivolol produced a decrease of the choroideal blood flow (-11,2 \pm 0,9%, p=< 0,05) and also no significant change of the ciliary blood flow. In all three series we could detect a clear decrease of IOP and heart rate.

Conclusion All tested betablockers showed vasoconstrictive effects on the choroidea. Although they are different in their nature they showed similar effects by intravenous administration on the examined parameters. A vasodilative effect of nebivolol could not be detected.

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A rat model to study choroidal blood flow

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Purpose To develop a model for the investigation of blood flow of in the choroid of rats. Due to their similarities in ocular blood supply und availability, rats are becoming increasingly interesting models in ocular blood flow research. The ability of choroidal autoregulation during changes in perfusion pressure has been shown in various species already. The presented study aims to show autoregulation in the choroid in a new model for blood flow measurement in the choroid of rats.

Methods Male Brown-Norway rats were anesthetised with Ketamin Hydrochloride (100 mg/kg) i.p.. A catheter for blood pressure measurement was inserted into the right femoral artery and advanced to the aorta, another catheter was inserted into the right femoral vein for drug administration. Pentobarbital was used to provide adequate anaesthesia for the following procedures, subsequently the animal was respired via a tracheotomy. Endtidal CO₂ was kept in the range between 35 – 45 mmHg and airway pressure was limited to 6 cm H₂O. A thoracotomy was performed through the 8th intercostal space and a hydraulic occluder was placed around the inferior vena cava just above its passage through the diaphragm. Choroidal blood flow was measured with a custom-made, non-invasive laser Doppler Flowmeter.

Results Blood pressure was reduced through a mechanical reduction of venous return, typically from 80 mmHg to 20 mmHg. Between 80 and 50 mmHg, blood flow remained virtually constant, between 50 mmHg and 20 mmHg, a linear relationship between blood pressure and blood flow was observed.

Conclusion The presented model is appropriate for the investigation of blood flow in the choroid of rats. Autoregulation in the choroid of rats could be demonstrated.

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Cis-urocanic acid suppresses UV-B-induced interleukin-6 secretion and cytotoxicity in human corneal and conjunctival epithelial cells in vitro



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Purpose Urocanic acid (UCA) is a major UV-absorbing endogenous chromophore in the epidermis and is also an efficacious immunosuppressant. The anti-inflammatory and cytoprotective effects of cis-UCA were studied in ocular surface cell cultures exposed to UV-B irradiation.

Methods Human corneal epithelial cells (HCE-2) and human conjunctival epithelial cells (HCEC) were incubated with 10, 100, 1000, and 5000 µg/ml cis-UCA with and without a single UV-B irradiation dose. Cell viability was measured by the colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.

Results The UV-B irradiation elevated IL-6 secretion by 7 to 9-fold in HCE-2 cells and by 2-fold in HCEC cells as analyzed with ELISA., declined by 30 to 50 % in HCE-2 cells and by 20 to 40 % in HCEC cells after UVB irradiation. Treatment with 100 µg/ml cis-UCA completely prevented both the elevated IL-6 secretion and the decrease in cell viability to the same level as nonirradiated control cells in both cell types, i.e. simultaneous anti-inflammatory and cytoprotective effects against UV-B radiation. No significant effects on IL-6 secretion or viability of the nonirradiated cells were observed with 10 and 100 µg/ml cis-UCA, while 1000 µg/ml cis-UCA evoked secretion of IL-6 in both cell types. The 5000 µg/ml concentration was toxic.

Conclusion Cis-UCA may represent a promising anti-inflammatory and cytoprotective treatment option to suppress UV-B-induced inflammation and cellular damage in human corneal and conjunctival epithelial cells.

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Retrolubar flow velocities in open angle glaucoma and their association with mean arterial blood pressure

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Purpose Previous experiments indicate that vascular dysregulation may play an important role in the pathogenesis of primary open angle glaucoma (POAG). Furthermore, evidence has been provided that reduced flow velocities as assessed with color Doppler imaging (CDI) are a risk factor for the progression of the disease. In the present study the association between retrolubar flow velocities as measured with color Doppler imaging and ocular perfusion pressure were compared in patients with POAG and healthy control subjects.

Methods 253 patients with POAG and 198 healthy subjects have been included in this study. Peak systolic (PSV), end diastolic (EDV) and mean blood flow velocity (MFV) in the ophthalmic artery, the central retinal artery and posterior ciliary arteries was assessed using Doppler ultrasound. Resistance Index was then calculated as (PSV-EDV)/PSV. Ocular perfusion pressure (OPP) was assessed based on measurements of systemic blood pressure and intraocular pressure.

Results Intraocular pressure was higher in glaucoma patients than in healthy controls (p < 0.01). All flow velocities were significantly reduced in POAG patients as compared to healthy control subjects (p < 0.01 each), whereas resistance index was increased in POAG. An association between blood velocities was found between OPP and blood velocities in both groups. This association was, however, higher in POAG patients than in the healthy control group.

Conclusion The present study indicates that there is a more pronounced association between retrolubar flow velocities and OPP in POAG patients than in healthy control subjects. This supports the hypothesis that vascular dysregulation is present in patients with POAG.

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Combined effects of aging and a pro-diabetic diet on retinal function in a murine model of aging of the human eye

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Purpose Improvement of life expectancy and changes in the dietary behaviour of developed populations are accompanied with the prevalence of diabetes and age-related ocular pathologies. Aging of the retina is characterized by accumulation of lipids at its basement. Meanwhile increased oxidative stress is one of the features of aging and diabetes. The goal of our study was to evaluate the effects of a pro-diabetic fructose-rich diet on the functioning of the retina in a murine model of aging of the human eye, the ApoB100,LDLR-/- mice.

Methods 4 month-old ApoB100,LDLR-/- and SF2J control mice were bred under either a standard chow or a fructose-rich diet (60%) during 6 months (n=6 in each group). The functionality of the retina was further evaluated by single flash electroretinography (ERG) under scotopic conditions to assess the global response of the retina. Flicker ERG was monitored by increasing flash intensity upon 10Hz-fixed frequency of the stimulus to specifically assess the sensitivity of rods and cones.

Results The fructose-rich diet significantly decreased a- and b-wave amplitudes of the retina to single flash ERG. This diminution was intensified in ApoB100,LDLR-/- mice compared to control mice: -55.5% vs -33.4% for the a-wave, -41% vs -38% for b-wave, respectively. The fructose-enriched diet impaired the sensitivity of rods by 0.3 log unit in both strains of mice.

Conclusion Oxidative stress triggered by a pro-diabetic diet promotes the deleterious effects of aging on the retina by reducing its functionality and impairing its sensitivity to light stimulus.

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Efficacy and safety of topical cyclosporine versus vehicle in vernal keratoconjunctivitis in children

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Purpose To evaluate safety and efficacy of topical cyclosporine with a cationic emulsion versus vehicle in vernal keratoconjunctivitis (VKC) in children.

Methods Vernal keratoconjunctivitis of 118 children (range 4 yo to 21 yo) diagnosed with giant papillae were randomized in 3 groups. During one month the first group was treated topically QID with cyclosporine 0,05%, or cyclosporine 0,1%, or with vehicle. For the next two months the treatment was similar except for the third group randomized to cyclosporine 0,05% or cyclosporine 0,1%. Patient subjective improvement (5-points ordinal scale) was the primary criterion. Resampling simulations have been carried out to generate success rate distributions. Objective evaluation was a secondary criterion with corneal fluorescein staining appreciation.

Results The sex ratio of VKC was 1:4.4. Seasonal forms represented 23,7% of patients. The topical application of cyclosporine was rated comfortable by more than 76% of the patients at D28 and at 3 months. At D28, all groups showed subjective improvement; however when expressed in term of success/no success, subjective improvement was obtained for 89,8%, 84,6% and 70,0% of patients respectively with cyclosporine 0,05%, 0,1% and vehicle. Using resampling simulations, both success rates of 0,05% and 0,1% dosages were statistically significantly different ($p < 0.01$) versus vehicle. Improvement ($p < 0.01$) in overall rating of objective signs and corneal fluorescein staining was shown with cyclosporine 0,05%, 0,1% versus vehicle.

Conclusion Treatment by cyclosporine with cationic emulsion formulation appears well tolerated and efficient in children with VKC. New larger studies are necessary to confirm these first results.

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Lymphocyte and markers of inflammation detection in the conjunctival epithelium of patients with dry eye by enhanced flow cytometry

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Purpose The aim of our study was to test the hypothesis that the use of cell culture medium can increase the number of cells obtained by impression cytology (IC) sampling of the conjunctiva in dry eye patients, and make the analysis of epithelial and lymphocyte cell populations with flow cytometry possible.

Methods IC specimens were collected in 15 normal subject and 15 dry eye patients. Samples collected from the right eye were placed in cell culture medium containing 10% foetal calf serum (FCS), and samples from the left eye in Phosphate Buffered Saline containing 0.05% paraformaldehyde (PFA). The number of cells was analyzed by flow cytometry in both groups. Samples collected from a group of 30 dry eye patients were placed in FCS and stained for the expression of CK19, CD45, CD3, HLA-DR, and analyzed by flow cytometry. A control group of 10 subjects was used as control.

Results The number of cells counted in the FCS samples was statistically increased in the normal (12791 ± 11350 events per minute) and dry eye group (23468 ± 15596) when compared to PFA samples (2031 ± 2336 , and 2608 ± 1814 respectively). In dry eyes the low number of cells in PFA samples made possible only the analysis of HLA-DR expression, while in FCS samples epithelial (CK19+), leukocyte (CD45+), and lymphocyte (CD3+) cell populations were characterized, other than analyzed for HLA-DR expression.

Conclusion This study indicates that using this new method of preservation can enhance flow cytometry analysis of epithelial and immune cells of the conjunctiva in dry eye patients, opening new scenarios in the comprehension of its pathogenesis and in testing new therapies.

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Ocular surface findings in patients with congenital aniridia



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Purpose to study the Schirmer's test, tear Break-up time, ocular tear ferning pattern test and impression cytology results in patients with congenital aniridia

Methods 25 eyes of 25 patients with congenital aniridia underwent Schirmer's test I, tear break-up time, ocular ferning pattern test and impression cytology to study the ocular surface characteristics. Clinical findings were correlated with the results of the aforementioned tests.

Results Aniridia related keratopathy was Grade 0 in 12%, Grade 1-A in 52%, 1-B in 20% and Grade 2 in 16% of eyes studied. Schirmers Test I was normal in 96% of eyes. Tear break-up time was abnormal in 75% of patients. Ocular ferning pattern tests were as follows: Grade 1 (20%), grade 2 (50%), Grade 3 (20%) and Grade 4 (10%). Conjunctival squamous metaplasia revealed: Grade 0 (23%), Grade 3 (35%), Grade 5 (4%). Conjunctival keratinization was mild in 35% and moderate in 15%. Significant correlation was found between conjunctival keratinization and the degree of Aniridia-related keratopathy. Significant correlation was also found between ocular ferning pattern test and conjunctival keratinization.

Conclusion Aniridia-related keratopathy is a muco-deficient and lipo-deficient, more than an aqueo-deficient dry eye disease. Simple tests of the ocular surface must be done early on to direct the right kind of dry eye treatment in these difficult cases.

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Sutureless and glue free conjunctival autograft in pterygium surgery

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Purpose To review the safety and efficacy of sutureless and glue free conjunctival autografting for the treatment of primary pterygium

Methods Retrospective case notes review of 8 consecutive patients undergoing primary pterygium excision performed by a single surgeon over a 1 year period. Excision of the pterygium was followed by reconstruction using suture and glue free conjunctival autograft.

Results Mean follow up was 14 months (range 12 to 24). There were no transplant dislocations, failures or recurrences (0 of 8 eyes) after one year. The average operating time was 14 ± 2 (SD) minutes. One patient had transient graft oedema. Visual acuity was unaltered in seven patients, one patient with significant pterygium-induced astigmatism gained 3 Snellen lines. No major complications occurred and all patients approved of their cosmesis at their final visit.

Conclusion Sutureless and glue free autologous conjunctival grafting for primary pterygium surgery appears safe and efficacious and may aid in preventing complications attributed to the use of foreign materials.

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Compare optical with photometry measures of tear film lipid layer

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Purpose Quantity of tear film lipids could be estimated optically by colours of interference. By photometric evaluation it may be measured. Therefore we compared both measures.

Methods Patients with dry eyes were observed by Tearscope and Meibometer 550. For tearscope we used the 4-parted scale of colours. Meibometry was performed with clean stripes, which have touched the tear film. >300 units are said to be normal. We observed the tear film by Schirmer I, Lissamin green and fluorescein staining, and the break up time. We correlated the measurements with Pearson Correlation.

Results 85 eyes of 53 patients were observed. Both measurements of the lipids showed positive correlations $R=67$. There was no Correlation between any lipid measurement and the watery and mucin Layer ($R=0,1 - 0,3$)

Conclusion The measurement of lipids is similar in both measurements. The optical evaluation needs longer experience, the Meibometry needs standards to find reproducible results. The lack of correlation between lipid layer results and the other results of the tear film observation may point at the special function of lipids independent of the other layers of the tear film.

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Evaluation of spatial contrast sensitivity after the instillation of diclofenac eye drops

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Purpose To evaluate if diclofenac eye drop instillation is related with spatial contrast sensitivity (CS) impairment.

Methods Thirty ophthalmologically healthy Caucasian individuals (--male, --female), aged from 20 to 59 underwent CS testing. The examination was repeated 20 and 40 minutes after the instillation of diclofenac eye drops unilaterally. The fellow eye served as control.

Results All the examined individuals had normal visual acuity, color vision and CS before the diclofenac drop instillation. Four of them complained of a temporary glare at the eye in which diclofenac was instilled. These four individuals had decreased CS in low spatial frequencies (1.5 & 3 cycles/degree), in the examination performed 20 minutes after the instillation. The CS normalized again in the third CS evaluation performed 40 minutes after the instillation.

Conclusion The temporary glare that affects visual performance of some individuals after diclofenac eye drop instillation is related with a temporary decrease of spatial CS in low frequencies. Within this time period of 40 minutes after the instillation of diclofenac, individuals who experience visual disturbances should avoid activities that demand high visual efficacy or postpone the instillation for a more convenient time in relation to the duration of glare they have experienced.

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Dextran 70 solutions for the intra-operative control of corneal hydration in rabbit corneas

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Purpose In terms of excimer laser ablation, the cornea is water bound on an organic matrix. Corneal hydration might affect the excimer laser ablation rate, which could affect the accuracy of correction. It was the purpose of the study to investigate the use of Dextran solutions of varying concentrations to control corneal hydration in rabbit corneas.

Methods The corneal epithelium was removed by means of a rotating brush from both eyes of 10 anesthetized pigmented rabbits. The Dextran 70 solutions, with concentrations ranging from 1 to 7%, was topically applied to the eyes for two minutes. The thickness of the cornea in each eye was determined by ultrasound pachymetry after epithelial removal and immediately after rinsing. The Dextran concentration corresponding to zero change in pachymetry was determined by linear regression. In a different series of experiments, rabbit corneas were either overhydrated by means of rinsing with saline or left exposed to dehydrate. Following this artificial change in hydration the corneas were rinsed for 2 minutes with the previously determined isotonic concentration.

Results A strong negative correlation between Dextran concentration and change in pachymetry was observed. Rinsing with the isotonic concentration (2.6%) regulated pachymetry within 2% of the original value.

Conclusion Corneal hydration can be regulated intraoperatively by means of appropriate Dextran solutions. This may prove useful in cases that hydration is potentially different from normal such as in the case of extensive surgical manipulations prior to laser ablation.

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A pocket slitlamp

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Purpose I here describe a prototype pocket slitlamp for students and non-specialists. It might help bridge the growing gap between ophthalmology (and optometry) and other specialties.

Methods MATERIAL Pocket microscopes, condensing lenses, modified pentorch and a platform. PILOT TESTS Evaluation and photographs of optical sections of 1) Anterior segment and lens of the eyes of the author and four volunteers. 2) Wrist watch crystals. Is a wristwatch a suitable model eye?

Results The prototype weighs 100g, costs EUR35 (£30) and can be carried in a pocket. The magnification was 0-30X, and the slits of light were 0.5-2mm wide. The digital camera cost EUR85 (£79.99). PILOT TEST RESULTS 1) Clinically adequate optical sections of the cornea, anterior chamber and lens were seen and photographed. 2) The outer and inner surfaces of the wristwatch crystal, watch face and the space in between were clearly seen. They simulated the corneal epithelium and endothelium, the iris and the anterior chamber. LIMITATIONS The view is monocular, there is no zoom and the light is less intense than the maximum of a conventional slitlamp.

Conclusion When developed this miniature slitlamp could help both in the instruction of students and in the clinical practice of non-specialists. Students could practice on each other and on wristwatches, which make good model eyes.

Commercial interest

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Simultaneous topo-guided photorefractive keratectomy (PRK) followed by collagen cross-linking (CXL) for the treatment of keratoconus

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Purpose To present the results after simultaneous topoguided Photorefractive Keratectomy (PRK) followed by corneal collagen cross linking with Riboflavin/Ultraviolet - A irradiation (CXL) for the treatment of keratoconus.

Methods In this prospective case series, 22 patients (27 eyes) with progressive keratoconus participated. All patients underwent customized topography guided photorefractive keratectomy (PRK) immediately followed by corneal collagen cross linking with the use of riboflavin and ultraviolet - A irradiation.

Results Mean follow up was 10.72±5.98 months (range from 3 to 19 months). Mean preoperative spherical equivalent (SE) (diopters, D) and defocus were $-3.03 \pm 3.23D$ and $4.67 \pm 3.29D$ while at the last follow up examination were significantly reduced to $-1.29 \pm 2.05D$ and $3.04 \pm 2.53D$, respectively. Preoperative mean (LogMAR) Uncorrected Visual Acuity (UCVA) and Best Spectacle Corrected Visual Acuity (BSCVA) were 0.99 ± 0.81 and 0.21 ± 0.19 while at the last follow up examination were improved to 0.16 ± 0.15 and 0.11 ± 0.15 respectively. Mean preoperative steepest meridian keratometry from $48.2 \pm 3.4D$ was reduced to $45.13 \pm 1.8D$ at the last follow up examination.

Conclusion Simultaneous PRK followed by CXL seems to be a promising treatment capable of offering patients a functional vision and halting progression of the ectatic disorder. Longer follow up and larger case series are necessary in order to fully evaluate this new innovative combined procedure.

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Comparison of morphometric and morphological OCT Visante and UBM potential in anterior segment imaging

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Purpose To compare anterior segment modalities such as Visante OCT and UBM as an important tool for assessing anterior segment examination.

Methods 166 patients (96 women and 70 men, mean age 54,32± 9,92 years) various pathologies in anterior segment area and after refractive and glaucoma surgery were examined with both OCT Visante (Zeiss) and UBM (QuantelMedical). In the above examination, we have compared sectorized b-scan image, made by 50Hz UBM head (patient in horizontal position) to scan image displaying anterior chamber (ac-scan), made by OCT Visante device (patient sitting).

Results Both devices promise quantitative information and imaging of corneal pathologies, keratoplasty results and follow up also anterior chamber imaging, direct angle visualization, lens pathologies, diagnostic and postoperative results of cataract and glaucoma. Bland&Altman analysis has shown, that results obtained by UBM in comparison to Visante OCT in anterior chamber depth measurement (AC) were averagely higher by 0.21mm (± 1.96 SD= 0.30) in case of PKP and 0.2mm (± 1.96 SD= 0.23) in case of lamellar keratoplasty. The results for filtration angle measurement, were averagely higher by 3.3 degrees(± 1.96 SD= 4.30) in case of PKP, and 3.4 degrees(± 1.96 SD= 3.9) in case of lamellar keratoplasty. Calculations obtained by ultrasound biomicroscope were slightly higher, however there was no significant statistic difference.

Conclusion Both used devices are helpful in clinical practice, however OCT Visante, with high resolution scans and as a non contact device with shorter examination time is more usefull in every day practice. Moreover UBM is an indispensable tool for anterior segment tumors involving ciliary body.

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Prospective evaluation of the Boston keratoprosthesis

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Purpose To assess the sustainability of the Type I Boston Keratoprosthesis in the developing world and report 18-month follow-up data on the International Boston Keratoprosthesis Study Group pilot project in Ethiopian.

Methods Through an international collaboration between the Massachusetts Eye and Ear Infirmary, USA and the Menelick II Hospital, Ethiopia, six pre-selected patients underwent surgery with th Type I Boston Keratoprosthesis (KPro). Patient inclusion criteria included hospital access, multiple graft failure, or binocular blindness. An International Protocol was developed to standardize evaluation and follow-up. To reduce costs, the patient's own cornea was used as a carrier for the KPro when possible instead of a donor cornea. Follow-up reports were transmitted electronically to all physicians involved.

Results Six Ethiopian patients received a Boston KPro under local anesthesia without complications. In two patients, their own cornea was used as a carrier for the KPro. All patients had improved visual acuity from a baseline vision of hand motions. Postoperative vision ranged from CF at 5m to 20/40. All patients were able to self-administer their ophthalmic medications and five of six patients reported greater independence in ADLs. There were no infections, or compliance issues.

Conclusion Early results indicate that the Boston KPro may be an option in the developing world. KPro complication rates were no different from developed countries. The use of the patient's own cornea was not associated with a poor outcome. It remains unclear as to whether KPro may be superior to PK in this setting. Careful patient selection, standardized protocol implementation, and meticulous post-operative follow-up are necessary for long-term sustainability.

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Retinal detachments after Boston keratoprosthesis: incidence, predisposing factors and outcomes of repair

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Purpose Knowledge of predisposing factors to retinal detachments in certain patient populations can guide surgical planning in Boston keratoprosthesis placement.

Methods This is a retrospective, noncomparative, interventional case series. A review of 166 patients (203 eyes) who underwent Boston Keratoprosthesis implantation at the Massachusetts Eye and Ear Infirmary from 1993 to 2008 was performed. Most patients had very advanced ocular disease. Predisposing factors to retinal detachment and details of timing after surgery were investigated. Anatomical and visual outcomes of retinal detachment repair were analyzed.

Results

Table 1. Retinal detachment after K-Pro implantation

Category	Number of Eyes	Eyes with RD
Autoimmune systemic disease	72	24 (33%)
Chemical burns	14	3 (21%)
Non-autoimmune etiology	117	10 (8.5%)

Eighty-nine (89%) of patients with retinal detachments have visual outcomes of less than 20/200 despite surgical repair. Majority of retinal detachments in all three subgroups occurred within the first six months after K-Pro placement.

Conclusion These data suggest that a retinal detachment following K-Pro implantation is more frequent in patients with autoimmune disease (where K-Pro is generally not recommended). When they do occur, they portend a poor visual prognosis. Patients with underlying autoimmune systemic disorders should be warned of the higher risk of developing retinal detachments after keratoprosthesis surgery and warrant close co-management with a vitreoretinal specialist.

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Cost-effectiveness of the Boston keratoprosthesis

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Purpose Blindness due to corneal disease accounts for approximately 8 million of the 37 million blind in the world. A 2007 study confirmed the global cost-effectiveness (CE) of cataract surgery and penetrating keratoplasty (PK). We sought to determine the CE of the Boston Keratoprosthesis.

Methods In a retrospective chart review, patients who underwent KPro type I surgery in the United States over the last 5 years were identified. Patients had to have a minimum of 2 years of visual acuity follow-up data. Patients with autoimmune diseases and chemical burns were excluded. Eighty-three patients were included. CE was determined by cost-utility analysis (CUA). Complications and additional procedures were incorporated into a weighted cost decision tree. A conservative retention estimate of 85% was assumed at 5 years. Visual acuity values were converted to utilities and determined to be normally distributed per Shapiro-Wilk testing.

Results The mean preoperative utility value was 0.449 increasing to 0.630 postoperatively. The mean incremental utility was 0.182. Amongst our cohort, 65 patients experienced improvement in their BSCVA at 2 years, 13 experienced no improvement, and 5 deteriorated. A total discounted incremental QALY gain of 0.763 was obtained. The total discounted cost associated with this utility equaled \$11250 (USD). The cost-utility of the KPro was \$13106 per QALY. The model was assessed using both univariate and multivariate sensitivity analyses.

Conclusion The KPro is becoming an established procedure in the US for complex ophthalmic patients, often with numerous comorbidities. The cost effectiveness of KPro was determined in this study to be \$13106 USD per QALY. This is comparable to PK, which has been reported in the range of \$12000-16000 USD per QALY.

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Boston keratoprosthesis with titanium back plate

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Purpose Following implantation of a Boston Keratoprosthesis (BKPro), formation of a retroprosthetic membrane (RPM) is an indication of postoperative intraocular inflammation. The purpose of this retroactive study has been to compare BKPro back plates made of titanium with the standard PMMA back plates. Any difference in RPM formation would guide our future choice of material for the device.

Methods The design was a retroactive analysis looking at the surgical outcomes after 6 months in patients who had BKPro implants. Pediatric cases were excluded. The indications for having a KPro implant were broadly divided into three categories: autoimmune (n=12), chemical burns (n=11), and others (non-autoimmune) (n=55). Three types of back plates were studied:

1. threaded (screw-on) PMMA (n=39),
2. non-threaded (snap-on) PMMA (n=16) and
3. non-threaded titanium back plates (n=23).

Main outcome measured was incidence of RPM formation at 6 months after surgery that warranted removal by either YAG laser membranotomy or surgery.

Results The percentage of patients who developed RPM was greatest in the threaded PMMA group (46%), followed by the non-threaded PMMA (31%), although this difference did not achieve statistical significance (p=0.5). The non-threaded titanium group showed the least RPM formation (13%), which was significantly less than both the threaded PMMA (p=0.002) and the threadless PMMA (p=0.043) groups.

Conclusion Titanium KPro back plates induce less RPM than PMMA back plates, threaded or non-threaded. It seems that the titanium plates in this setting are more tissue friendly and cause less inflammation than PMMA.

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Comparison of the visual response to corneal enhancement on femtosecond and microkeratome created flaps.

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Purpose To evaluate the evolution of visual acuity after enhancement after LASIK performed either with a mechanical microkeratome (MM) or femtosecond laser (FS), up to the third month post-op.

Methods Retrospective analysis of 148 consecutive eyes who had LASIK, 125 with femtosecond laser (intralase) and 123 with a mechanical microkeratome (Moria M2), had an enhancement performed between the third and the sixth month post-op. All the surgeries were performed by the same surgeon. All the patients were examined preoperatively, 24 hours, 7 days, one month and three months after the enhancement.

Results Both groups had good visual outcomes with no significant differences in visual acuity at the third month. The uncorrected visual acuity (UCVA) was 1.02 ± 0.2 in both groups (p=0.9), the best corrected visual acuity (BCVA) was 1.07 ± 0.1 and 1.09 ± 0.1 for the FS and the MM groups respectively. The safety (1.02 ± 0.1 in the FS group, 1.03 ± 0.1 in the MM group, p=0.7) and efficacy (0.97 ± 0.1 and 0.95 ± 0.1 for the FS and MM respectively, p=0.4) were similar in both groups. Nevertheless we found a statistically significant difference on UCVA on the first week (0.93 ± 0.1 femtosecond vs 0.98 ± 0.1 microkeratome, p=0.01).

Conclusion Visual acuity recovery is slower after enhancement on FS flaps only on the early postoperative period. Probably, there is an increased epithelial damage or a superior inflammatory response due to a more difficult flap-lifting in FS vs a MM cases.

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Comparison of deep anterior lamellar keratoplasty results in different TGFβ1 corneal dystrophies

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Purpose To compare the results of deep anterior lamellar keratoplasty in eyes with TGFβ1 corneal dystrophies.

Methods 10 eyes of 8 patients with TGFβ1 corneal dystrophies after DALK were recruited for the study. There were 5 eyes with LCD1 (R124C), 2 eyes with atypical LCD (H626R), 2 eyes with TBCD (R555Q), and 1 eye with GCD1 (R555W). Main outcome measures were: complication noticed during Descemet membrane air dissection, 6 month graft and stromal bed clarity, presence of stromal bed folds during OCT exam, best corrected visual acuity (BCVA), corneal astigmatism and endothelial cell density (ECD).

Results Local Descemet membrane perforation during DALK occurred in 1 eye with R555W mutation and 2 eyes with H626R mutation. Descemet membrane detachment was diagnosed postoperatively in one eye with R124C mutation. DMD resolved spontaneously within one month. At 6 month all grafts reminded clear, but there were thick stromal bed folds visible during OCT exam in 1 eye with R555W mutation and 2 eyes with H626R mutation. BCVA was 0.8 in eyes with R555Q, 0.4 in eyes with R124C, 0.2 in eyes with H626R and 0.3 in eye with R555W mutation. There were no differences noticed in corneal astigmatism or ECD at 6 month. Mean corneal astigmatism was $3.3 D (\pm 0.43)$ and ECD was $2156 \text{ cells/mm}^2 (\pm 758)$.

Conclusion The TGFβ1 dystrophy type influences on the results after DALK. TBCD and LCD1 with no deep stromal or Descemet involvement are good candidates for DALK.

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Refractive and biomechanical results of intrastromal ring segments for keratoconus

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Purpose To analyse the refractive and biomechanical changes in the cornea after implantation of intrastromal ring segments.

Methods The study included 43 eyes of 36 patients (mean age 38 years); 7 eyes had received Intacs® and 36 KeraRings®. Visual acuity, spherical equivalent, K readings, corneal hysteresis (CH) and corneal resistance factor (CRF) were measured pre-op, at 6 and at 12 months post-op. CH and CRF were derived from the Ocular Response Analyser (ORA).

Results UCVA improved from 1.10 logMAR to 0.65 at 6 months and 0.62 at 12 months post-op. BCVA improved from 0.32 logMAR to 0.25 at 6 months and 0.22 at 12 months post-op. Spherical equivalent improved from -5.28 to -1.30 at 6 months and -1.85 at 12 months post-op. Sim K improved from 5.81 to 3.64 at 6 months and 4.10 at 12 months post-op. Mean K improved from 48.9 to 46.3 at 6 months and 46.7 at 12 months post-op. All changes between pre-op and 6 months post-op were significant, but between 6 months and 12 months they were not. Pre-op CRF (mean 6.37) and CH (mean 7.34) did not change significantly up to 12 months post-op.

Conclusion Intrastromal ring segments for keratoconus reduced the spherical equivalent refraction and improved the UCVA and BCVA at 6 months post-op and remained stable up to 12 months. ORA's biomechanical factors CH and CRF did not change up to 12 months post intrastromal ring segment implantation.

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Descemet's membrane detachment diagnosis using a very high frequency ultrasound scanning system (Artemis 2, Ultralink LLC)

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Purpose To present a case report of Descemet's Membrane Detachment after cataract surgery diagnosed using a Very High Frequency ultrasound scanning system (Artemis 2, Ultralink LLC).

Methods A 74-year-old female was referred to our institute due to persistent corneal edema after cataract surgery. Slit lamp examination revealed severe total corneal edema (obscuring anterior chamber and iris). Using a very high frequency (VHF) ultrasound scanning system (Artemis 2, Ultralink LLC) Descemet's Membrane Detachment (DMD) was diagnosed.

Results The patient was treated with anterior chamber air tamponade using a 27 gauge cannula attached to a syringe with a filter riled under topical anesthesia. Descemet's membrane was successfully reattached and corneal edema resolved after two weeks.

Conclusion This case report demonstrates that the VHF ultrasound scanning system (Artemis 2, Ultralink LLC) could be a useful instrument for the early recognition and possible management of DMD after cataract surgery, especially in patients in which diagnosis is difficult due to significant corneal clouding.

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Visual fields after refractive surgery

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Purpose Laser correction of ametropias is achieved by changing the radius of curvature of a central portion of the cornea. Although current refractive laser technology allows relative uniform correction across an optical zone larger than the scotopic pupil it must be kept in mind that centration of the ablation pattern pertains only to objects on the line of sight. Off-axis objects are imaged not only through the corrected portion of the cornea but additionally through a meniscus of paracentral cornea involving the transition zone as well as untreated cornea. It was the purpose of this study to investigate the role of this optical effect in the peripheral visual fields in patients undergoing photorefractive keratectomy.

Methods Twenty one eyes of thirteen patients were evaluated before and 3 months following PRK for the correction of myopia (average: -3.4D, SD:2.4D) at an optical zone of 6.5mm with the Allegretto 400 Excimer laser. Evaluation of the optical fields was made using the MEDMOND M700 visual fields analyzer using a full threshold procedure. Measurements were performed under cycloplegia while in all cases the subjects were corrected for the distance of the stimulus (+3 D)

Results The detection threshold exhibited a decrease ranging from 0.5 to 9 dB (mean:1.5 dB, SD:2.5) for the periphery of the optical field (30 to 40 degrees). This change was statistically significant ($p=0.00628$). No statistically significant difference was observed for the central visual field (0-22 degrees).

Conclusion The loss of peripheral visual fields following refractive surgery may be attributed to the deteriorated retinal image quality in the peripheral visual field. A mathematical model of this reduction is proposed

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Corneal wavefront aberration measured by a rotary scanning system

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Purpose Corneal wavefront aberration measurements are important in many areas such as refractive surgery, diagnosis and management of corneal disease as well as to understand the eye's optical system. In this work the authors present the corneal anterior and posterior surface wavefront aberrations measured by an optical corneal tomographer that uses two Scheimpflug cameras attached to an innovative illumination system that allows a rotary scanning of the entire cornea.

Methods The corneal wavefront aberration was computed from the corneal topographic height data of both corneal surfaces obtained by an innovative rotary scanning system. The topographic height data was computed from corneal optical sections obtained by illumination with a collimated beam expanded in a fan by a small cylindrical lens; this lens is provided with motor driven rotation in order to perform automated rotary scan of the whole cornea. This system has two stationary Scheimpflug cameras whose images are combined as if there was one virtual rotary camera synchronized with the cylindrical lens.

Results After appropriate processing of the cameras images it is possible to produce true elevation maps and consequently, to compute wavefront aberrations of both corneal surfaces. In this work we present corneal aberrations measured with this system and explained how this is achieved.

Conclusion This new optical system allows the measurement of corneal wavefront aberration as well as corneal thickness and 3-D mapping of both corneal surfaces.

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Optical qualities of the Boston keratoprosthesis

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Purpose To evaluate the optical characteristics of the Boston Keratoprosthesis (KPro) in relation to visual function. To determine glare sources and evaluate possible glare control. To explore the feasibility of fundus imaging. To examine the binocular consequences of implanting the device when the fellow eye has normal vision.

Methods The computed and optical bench measured point spread function and corresponding modulation transfer function (MTF), as well as glare sources, were compared. A model eye construct was used to determine the relative impact of different sources of glare and its control. The effect of glare in eyes implanted with a KPro was measured with a Brightness Acuity Test (BAT) with and without a dark iris contact lens (dCL) for 8 eyes with Type I and 2 eyes with Type II KPro. Computed and measured visual fields were compared. The power of a lens that provides a wide field view of the fundus was calculated. Images with a fundus camera and lenses were obtained. Stereopsis was measured in 5 Type I KPro patients with an intact fellow eye.

Results The computed and measured MTF for the KPro were found to be very close to the diffraction limit. Both the model eye and patients testing identified the hazy corneal graft as the main source of glare, which was controlled with a dCL. In 3 patients with an intact fellow eye, modest stereo acuity was gained with KPro implantation. Binocular visual field is not expanded when implanting a patient with normal fellow eye. Good image quality of the fundus is achieved in eyes with clear media.

Conclusion Disability glare can be reduced with the use of a dCL. Implanting the KPro in a patient with an intact fellow eye only marginally improves visual function. Adequate fundus imaging can be obtained.

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Reliability and interobserver variability of non-invasive tear film parameters

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Purpose To summarize our experiences with a non-invasive tear interference device.

Methods In thirty-seven subjects, we measured non-invasive tear break up time (NIBUT) three times, pictured the lower tear meniscus height (TMH) and the tear film lipid layer pattern five times after complete blinks with Keeler Tearscope Plus. Three independent observers evaluated the photos. Additionally, the lipid pattern was graded by the observers with real-time examination in thirty-one subjects. We analysed the repeatability of the tear parameters after different blinks, and the agreement between the observers on assessments of the photos and real-time examination.

Results The results measured after different blinks did not show significant differences in case of any tear film parameters. The intraclass correlation coefficient (ICC) with 95% confidence interval of NIBUT and mean TMH were 0.605 (0.428-0.754) and 0.901 (0.848-0.942), respectively. There was significant difference between the measured TMH of the observers ($p < 0.001$). The results showed significant correlations ($r \geq 0.912$, $p < 0.001$), but the standard deviation of the three measured TMH was meanly 11.13% of the mean of them. The graded lipid patterns of observers showed significant differences ($p = 0.01$) in case of photos, but did not ($p = 0.137$) during real-time examination.

Conclusion The Tearscope Plus is a useful tool of non-invasive tear film examination. However, the evaluation of the parameters is partly subjective, which reduces the reliability of the methods. The real-time examination of lipid pattern is recommended, because the kinetics of lipid layer allows additional information for grading, therefore decreasing the interobserver variability.

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Wavefront analysis in unilateral herpetic keratitis

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Purpose To assess optical aberrations using wavefront analysis in the affected eye of patients with unilateral herpetic keratitis with no apparent visual disturbance (visual acuity: 20/20), and to compare these results with those of the non-affected eye

Methods Corneal optical aberrations from 5 patients with unilateral herpetic keratitis and normal visual acuity (20/20 OU) have been assessed using dynamic skiascopy with OPD Scan II[®] (Nidek[®]). Both eyes in all patients were apparently normal using slit-lamp examination, and wavefront analysis was performed in a quiescent period of the herpetic disease. The fellow eye was used as control. Data were retrospectively analyzed by a blinded examiner.

Results Zernike's polynomials and MTF curve analysis showed bilateral abnormalities in 4 patients, with no specific difference between the two eyes. In contrast, one patient had wavefront disturbances in the affected eye, whereas the non-affected eye was normal.

Conclusion Moderate herpetic keratitis may induce wavefront abnormalities in some patients despite a normal visual acuity and an apparently normal cornea. This could explain visual discomfort that is sometimes described in such patients. Further studies are needed to understand wavefront aberrations induced by herpetic keratitis with normal examination.

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Ocular Response Analyzer waveforms analysis in normal and post-laser in situ keratomileusis eyes

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Purpose Many clinicians have noticed that the morphological signal produced by Ocular Response Analyzer (ORA) (Reichert) is a unique "signature" for the eye being measured and suggested that probably there is other valuable information contained in these signals. This study explores the possibility of using information contained in these waveforms comparing them between normal and post-LASIK eyes.

Methods Corneal biomechanical properties of 40 normal eyes and 20 post-LASIK eyes were obtained with ORA. Corneal thickness was measured with the SP-100 Handy pachymeter (Tomey). Data acquired by ORA was exported to an Excel worksheet to obtain parameters such as areas of both the applanation and pressure signal curve, area of the "in" and "out" applanation curves, maximum applanation peaks, maximum of the pressure signal.

Results There were significant differences between normal eyes and those submitted to LASIK for almost the studied parameters. Normal eyes show a bigger applanation curve area ($p < 0.001$); the first peak maximum as well as its area are higher than for post-LASIK corneas ($p < 0.001$). For the second peak there are no statistically significant differences between normal and post-LASIK eyes. Areas of both applanation peaks shows no significant differences for the post-LASIK eyes ($p = 0.794$) contrary to that observed in normal eyes. Post-LASIK eyes show a lower maximum for the pressure signal curve and a smaller area for the second half of the pressure curve than for the normal eyes.

Conclusion The results obtained indicate that there is more information in ORA measurements than have been used. Some of the corneal biomechanical properties studied, as well as waveforms, have not the same behavior in normal and in post-LASIK eyes.

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Emergency keratoplasty

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Purpose Retrospective study about emergency keratoplasties made in our Service of Ophthalmology between 2006 and 2009.

Methods From the total number of 92 penetrating keratoplasties (PKP) 12 were done as an emergency. Of these, 66,7% were women and 33,3% men. Causes leading to surgery, donor and receptor cornea and eye, graft size, surgical technique, postoperative evolution, visual acuity and graft transparency were analysed.

Results Range of age was between 25 and 78 years. Corneal perforation was present in 8 cases. Corneal pathologies were: trophic and infectious corneal ulcers of bacterial, herpetic and fungal origin, bullous keratopathy and post-LASIK hydrops. Best corrected visual acuity was between 0,1 y 0,7. Postoperative complications were rejection of the graft (1 case), relapsed ulcer (3 cases), infections and calcium deposits. 9 grafts still remain transparent.

Conclusion Emergency keratoplasties have worse prognosis, as the corneas suffer from an active inflammation and/or infection, including a possible perforation. Alternatives for penetrating keratoplasty are corneal adhesives, lamellar keratoplasties and the use of amniotic membrane.

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Corneal biomechanics study: relationship between corneal aberration – hysteresis

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Purpose To determinate the relationship between corneal aberration and a bio-mechanical parameter: the corneal hysteresis.

Methods 122 eyes of 61 patients were included in a prospective and transversal study. Range of age was between 18 and 60 years. The following measurements were realised: Corneal topography with KERATRON (Optikon) to obtain the root mean square (RMS) of corneal aberration and measurement with Ocular Response Analyzer (ORA) (Reichert) to obtain the Corneal Hysteresis (CH). The correlation between RMS – CH was calculated by SPSS 12.0 (Statistical Package for the Social Sciences, Chicago, IL) using the correlation of Pearson for quantitative and independent variables.

Results No statistically significant correlation could be demonstrated between RMS and CH analysing both eyes as well as left and right eyes separately.

Conclusion The relationship between CH and each Zernike coefficient separately could be analysed as other studies found a correlation between intraocular pressure and high order aberrations. The importance of corneal biomechanical parameters like CH and CRF, values determined by the ORA, is not well defined yet; however the influence of these factors not only on intraocular pressure but also on corneal pathologies should be considered.

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Tear level of nerve growth factor in normal dogs

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Purpose To evaluate tear level of nerve growth factor (NGF) in normal dogs.

Methods In the present study we investigated the presence and the level of NGF in the tear of 12 normal dogs (24 eyes) with different sex (5 males and 7 females) and age (ranging from 2 to 12 years old). Tears were collected by washing the eye surface 150 microliter of physiological salt after clinical examinations. Tear samples were stored at -20°C until tested for presence of NGF performed with a commercially available immunoenzymatic assay and expressed as pg/ml of sample.

Results All samples examined showed presence of NGF, suggesting that this growth factor is a constitutive biological mediator of the ocular surface. It was also found that the level of NGF in the right eye is different from the left eye. Thus, the average concentration in the tear of the right was 7,97 (ranging from 5.0 to 38.3 pg/ml), whereas the average in the left eyes was 15,79 (ranging from 5.0 to 67.5 pg/ml).

Conclusion Tear fluid is a secretion of lacrimal gland that lubricates the eye surface and plays a critical role in response to eye irritation, by improving optical performance and the deficit induced by keratoconjunctivitis sicca. The observation that NGF is present in tear of dogs in a physiological relevant amount suggests that this molecule may be involved in the regulation of corneal homeostasis. Moreover, as NGF is known to play a role in emotional and anxiety-like behaviour, and tear secretion is a widespread phenomena among vertebrates, the possibility exists that NGF might be also involved in these behaviour responses. Because cats rarely suffer from emotional behavioural and keratoconjunctivitis sicca further studies may help to understand the role of NGF in tear secretion.

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Corneal biomechanics study (1): Central corneal thickness versus corneal resistance factor

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Purpose Study about the biomechanical characteristics of the cornea.

Methods We studied 122 eyes of 61 patients (33 women and 28 men) selected prospectively and consecutively. Range of age was between 18 and 60 years. The following determinations were carried out: Corneal topography with KERATRON (Optikon), measurement with ORA (Reichert) and ultrasonic pachymetry. The correlation between CCT - CRF was calculated by SPSS 12.0 (Statistical Package for the Social Sciences, Chicago, IL) using the correlation of Pearson for quantitative and independent variables.

Results We found after statistical analysis of data, a statistically significant correlation between the corneal resistance factor determined by the ORA, and central corneal thickness measured by ultrasonic pachymetry. The results are similar analysing both eyes as well as left and right eyes separately.

Conclusion The importance of corneal biomechanical parameters like CH and CRF, values determined by the ORA, is not well defined yet; however the influence of these factors not only on intraocular pressure but also on corneal pathologies should be considered.

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Istopathological aspects of complications of DSAEK: our experience

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Purpose Descemet-stripping automated endothelium Keratoplasty (DSAEK) is a selective tissue transplanation procedure in which only the deseaded endothelium and descemet membrane is replaced. The aim is to study the histopathologic features of DSAEK graft removed after graft failure and to correlate with relevant clinical features.

Methods Retrospective study of 2 cases of DSAEK graft failure in 22 patients who underwent DSAEK for Fuchs Endothelial Dystrophy and Bullous keratopathy. Perioperative clinical finding were correlated with pathologic finding of the explanted graft specimens. Anomalities in the DSAEK graft and in the interface between the recipient cornea and the DSAEK graft were studied.

Results All DSAEK procedures were uneventful. All graft failure occurred in association with graft detachment or more surgical manipulation. In 1 case the donor tissue detached in CA, in 1 cases the graft remained attached but failed to clear. By lighth microscopy, explanted donor tissue showed the presence of extensively damaged keratocytes in the deep posterior stroma and also in the interface at the graft- host junction. Histopatologic evaluation showed marked corneal edema and endothelial cell loss.

Conclusion The DSAEK proves to be a viable surgical technique in cases of imbalance corneal from endothelial problems. Rare are the cases of failure, in which histopathological study shows that the complication is due to bullous detachment between the two flaps, because of failure histopathological and clinical surgery

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Immune regulatory effect of rapamycin and cyclosporine A on experimental corneal allograft survival

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Purpose To analyze the efficiency of low dose systemic treatment with rapamycin (Rapa) alone or in combination with cyclosporin A (CsA) in preventing corneal allograft rejection in a high responder strain combination.

Methods A total of 80 C57BL/6 mice received corneal grafts of BALB/c donors. Recipients were treated with CsA 3mg/kg/day or Rapa 0.5mg/kg/day monotherapy or received combined treatment. Intraperitoneal injections were started on the day of surgery and continued for 14 days. In addition, corneal samples were subjected to real time RT-PCR analysis for cytokine transcription. The frequency of CD4+CD25+Foxp3+ T regulatory cells (Treg) in secondary lymphoid organs were measured by flow cytometry. Memory T cells were estimated by Elispot.

Results Monotherapy with Rapa significantly delayed allograft rejection ($p=0.03$). However, the combination of low dose Rapa and CsA prolonged corneal allografts survival at a significantly higher level ($MST=17.1 \pm 1.37$ days, $p=0.000004$). Addition of CsA to Rapa resulted in down-regulation of intragraft CD3, IL-2, IFN- γ and IL-10 transcription ($p=0.028$, $p=0.027$, $p=0.028$ and $p=0.027$ respectively). Rapa alone increased the frequency of CD4+CD25+Foxp3+ Tregs in draining lymph nodes, whereas addition of CsA reduced Tregs. Rapa monotherapy as well as combined treatment prevented development of alloantigen specific IFN- γ producing memory T cells in spleen.

Conclusion Combined treatment with low dose CsA and Rapa resulted in superior graft survival and effectively modulated mRNA expression of inflammation and infiltration markers. Supported by DFG (150/14-2) and SFB650 TP14

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Effect of mitomycin C on the cicatricial corneal response on laser-assisted subepithelial keratectomy to correct myopia

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Purpose To evaluate the influence of mitomycin C (MMC) on the predictability of refractive results after myopic laser-assisted subepithelial keratectomy (LASEK).

Methods This retrospective study comprised 1020 consecutive eyes that had LASEK to correct myopia. MMC 0.02% was applied for 30 seconds over the ablated cornea in all cases. Due to the use of MMC, the programmed ablation was 10% less than the intended correction to avoid overcorrection. The relationship between preoperative spherical equivalent (SE) and the 3-month postoperative SE was analyzed by lineal regression. We also compared the refractive result in the quartiles with the lowest and the highest preoperative myopia.

Results Preoperatively, the mean SE was -5.29 ± 2.6 diopters (D). 3 months post-op, the mean residual SE was $+0.16 \pm 0.4$ D in the lowest quartile (≤ -3.25 D) and -0.32 ± 0.8 D in the highest quartile (≥ -7 D) ($p=0.0001$). The mean cylinder was -0.27 ± 0.4 D in the lowest quartile and -0.41 ± 0.5 D in the highest quartile ($p=0.003$). There was a significant ($p=0.0001$, $r=0.15$) correlation between the preoperative and postoperative SE (i.e., the higher the preoperative SE, the greater the undercorrection).

Conclusion MMC seems to influence the spherical refractive outcomes after myopic LASEK. Using a 10% fixed hypocorrection, LASEK with MMC tends to overcorrect low myopic defects and undercorrect high myopias.

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Surveillance cultures of contact lenses of patients with Boston KPRO type keratoprosthesis

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Purpose Patients with Type 1 Boston KPRO require the use of a contact lens to avoid complications of drying of the corneal tissue surrounding the KPro. Additionally, these patients require chronic use of topical antibiotics to reduce the ever-present risk of endophthalmitis. This may promote the growth resistant organisms. The purpose of this study is to perform surveillance cultures of contact lenses of KPro patients to determine if organism growth may be detected prior to clinical presentation of infection.

Methods Cultures of contact lenses from Boston KPro type 1 patients were performed. All patients were maintained on a prophylactic antibiotic regimen including vancomycin. All contact lenses were removed 1 week to 1 month after insertion. Removal prior to 1 month was necessary when excessive protein deposits were noted on the lens. Lenses were removed cultured and analyzed under electron microscopy.

Results 45 patients with a KPro implanted in the last 18 months, the lenses of 15 patients were analyzed in the above manner. These 15 patients had various conditions including Stevens Johnson Syndrome, chemical burns, keratoconus, and multiple graft failures. Of the 15 lenses analyzed, one patient had cultures positive for streptococcus pneumonia, consistent with the patient's presentation of endophthalmitis. Three had cultures positive for coagulase negative staphylococcus although they did not present with any signs or symptoms of infection.

Conclusion Surveillance cultures appear to be an effective way to monitor the contact lenses of Boston KPro patients for possible organism growth. The detection of these organisms prior to the clinical presentation of infection could serve as a signal to change the patient's antibiotic regimen.

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Limbal choristomas – analysis of surgical technique and postoperative results (3 case reports)

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Purpose To present three case reports of the limbal choristomas. Analyze surgical technique, pathology slides, evaluate postoperative results by anterior optical coherence tomography (OCT) and keratotopography.

Methods Three patients with limbal choristomas were operated in Vilnius University Hospital. Location of the tumor was typical – inferiortemporal part of the limbus. All patients were admitted to the hospital lately after the time of diagnosis establishment. Patients were operated at the age of nineteen, six and fifteen years old. Case 1. Surgery was performed on the right eye of the nineteen years old lady. Visual acuity (VA) before the surgery 0,2. Case 2. Surgery was performed on the left eye of the six years old girl. VA before the surgery 0,8. Case 3. Surgery was performed on the right eye of the fifteen years old boy. VA before the surgery 0,2. Surgical technique-excision of the limbal tumor and lamellar corneal graft transplantation.

Results In case 1 and case 3 visual acuity of the operated eyes remained the same as it was before the surgery. In case 2 postoperative visual acuity improved by 2 lines. Excised tumors were evaluated by pathologist and diagnosis of the limbal dermoid was established. Postoperative results were evaluated by anterior (OCT) and keratotopography.

Conclusion 1. In purpose to prevent amblyopia scheduling of the surgery should be decided depending on the general health of the child and size of the tumor. 2. Appropriate surgical technique should be chosen after the evaluation of the size and location of the limbal dermoid. 3. Modern diagnostic tools such as OCT and keratotopography are important for the evaluation of postoperative results.

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Crosslinking by riboflavin and UVA. An electron transmission microscopy study in rabbits. Preliminary results

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Purpose Collagen crosslinking of the cornea (CXL) has been developed recently as a promising treatment of keratoconus. In vitro measurements have demonstrated a significant increase in biomechanical stiffness of the crosslinked cornea. The aim of the present study was to evaluate the effect of this procedure on the collagen fiber diameter of the rabbit cornea over time.

Methods The corneas of 10 New Zealand White albino rabbits were crosslinked by application of the photosensitizer riboflavin and exposure to UVA light (365 nm, 3 mW/cm²) for 30 minutes. The fellow control eyes were left untreated. On ultrathin sections of samples from the central and peripheral cornea, the collagen fiber diameter were measured with the help of morphometric computer software.

Results In the anterior stroma, 48 hours after the CXL the collagen fiber diameter in the treated corneas was significantly increased by 10% compared with the control fellow eyes. Four months after the CXL, the mean collagen fiber diameters were decreased as compared to the 48 h specimens. In the 6 month specimens the diameter of the collagen fibers was further decreased as compared to the control fellow eyes up to -8%.

Conclusion CXL leads to a significant increase in corneal collagen diameter 48 hours after the procedure. Interestingly, 4 months after the CXL, there is a decrease in the mean diameter of the collagen fiber and at 6 months, there is a further decrease up to 8% compared to the fellow eye. We can assume that the rigidity and the biomechanical stability of the cornea after CXL is increased because of the new intercollagen connections and collagen fiber diameter has little or no role in this procedure.

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Photorefractive keratectomy FAREK in Chinese patients with myopia

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Purpose To estimate the visual and refractive outcomes of simultaneous bilateral PRK (FAREK) for the correction of difficult cases of high myopia with steepest and very thin cornea in Chinese patients.

Methods We studied a series of 38 eyes of 19 patients who underwent simultaneous bilateral PRK (FAREK) for the correction of myopia with the sphere up to -15.6 D and the cylinder to -2.75 D in difficult cases with the steepest and very thin cornea. Coefficient K2 was up to 49.0D and central corneal thickness was 504.3±33.5 microns. Simultaneous bilateral PRK by using Mirtomicin C, soft contact lenses, cooling effects was performed by Russian surgeons with the NIDEK EC-5000. Follow-up period was up to 3 months. The age of our patients was 25.2±5.3 years. Pre-op UCVA and BCVA were 0.12±0.10 and 0.94±0.14 consequently.

Results Day of epithelization was 3.3±0.8. Post-op UCVA was 0.48±0.18 in the epithelization' day, 0.63±0.21 in the first week, 0.79±0.25 in the first month and 0.89±0.25 in the third month after FAREK. Pre-op IOP was 13.9±2.9 mm Hg. Post-op IOP was 8.2±1.8 in the epithelization' day, 7.8±1.6 in the first week, 9.7±2.2 in the first month and 10.0±2.3 in the third month. UCVA was equal or more than 1.0 in 2.6% in the epithelization' day, 13.8% in the first week and 41.6% in the first month after FAREK. There were no serious complications including hypertensions in all cases.

Conclusion We called our method FAREK – fast recovery excimer keratectomy. FAREK is a safe, reliable and effective method for the correction of difficult cases of high myopia with steepest and very thin cornea in Chinese patients. None of the authors has no financial interest in this work.

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LASIK surgery for myopia in Chinese patients

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Purpose To investigate the results of LASIK surgery for myopia in Chinese patients in Joint Russian-Chinese Ophthalmic Center.

Methods We reviewed our consecutive 296 cases (148 Chinese patients, including 92 women and 56 men) of LASIK surgery for myopia with the sphere from -1.0 to -13.5 D and the cylinder till -4.5 D. LASIK was performed by Russian surgeons with the NIDEK EC-5000 excimer laser and microkeratomes LSK Evolution-2 ("Moria"). Follow-up period was up to 3 months. The age of our patients was 26.7±6.2 years. Pre-op UCVA and BCVA were 0.13±0.08 and 0.97±0.08 consequently. All patients had standard ophthalmic examinations, including pneumotometry, refractometry etc. We performed statistical analysis by using "Statistica" program.

Results Ablation depth was 89.3±24.5 microns, ablation time was 47.53±15.26 sec. Post-op UCVA was 0.85±0.24 in the first day, 0.97±0.23 in the first week, 1.05±0.20 in the first month and 1.10±0.11 in the third month after LASIK. Pre-op IOP was 15.6±2.9 mm Hg. Post-op IOP was 10.2±2.7 in the first day, 10.5±2.5 in the first week, 10.7±2.9 in the first month and 9.1±2.6 in the third month. UCVA was equal or more than 1.0 in 51.0% in the first day, 71.1% in the first week and 79.1% in the first month after LASIK. There were no serious complications in all cases.

Conclusion The results of LASIK surgery for myopia in Chinese patients in the Joint Russian-Chinese Ophthalmic Center were effective and successful. LASIK surgery by using NIDEK EC-5000 excimer laser gives predictable and good results in Chinese patients. None of the authors has no financial interest in this work.

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Impact on visual function from light scattering and glistenings in intraocular lenses

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Purpose To investigate the impact on visual function from light scattering and glistenings in intraocular lenses (IOLs) in patients who had cataract surgery 10 years previously.

Methods 103 patients, who had phacoemulsification with implantation of Acrysof MA60BM IOLs 10 years previously, were evaluated with best corrected visual acuity (VA), and low contrast visual acuity (LCVA) 10% and 2.5%. The light scattering from the IOLs was measured by Scheimpflug photography. The degree of glistenings was also quantified at the slit-lamp. Eyes with coexisting pathology that could affect VA and LCVA were excluded.

Results The patients were divided into various groups according to the degree of light scattering and grade of glistenings. In two subsets of patients paired data from the patients' two eyes were analyzed. It was not possible to detect any significant impact on visual function, BCVA and LCVA 10% and 2.5% in eyes with a more pronounced light scattering or a higher grade of glistenings seen at the slit-lamp. The correlation between IOL dioptric power and both the total light scattering of the IOL, and the subjective grading of the intensity of the glistenings at the slit-lamp was statistically significant ($r=0.25$; $p=0.012$; $rS=0.23$; $p=0.019$, respectively).

Conclusion Most patients in this case series operated 10 years previously had severe glistenings and a high level of light scattering from their intraocular lenses. No detectable impact on BCVA, LCVA 10% and 2.5% was found.

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Coenzyme Q10 prevents human lens epithelial cells from light-induced apoptotic cell-death by reducing oxidative stress and stabilizing BAX/Bcl-2 ratio



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Purpose Cataract is one of the most prevalent eye disease and a major cause for legal blindness in the world. Beside others, cumulative light-exposure and apoptotic cell death are significantly associated with cataract development. In contrast, supplementation with antioxidants has been suggested to prevent premature cataractogenesis. This study investigates possible protective effects of Coenzyme Q10 (CoQ10) regarding light-induced stress and apoptotic cell death in human lens epithelial cells (LEC).

Methods Human LEC were either pre-incubated with CoQ10 or not and then exposed to white light. After 10 to 40 minutes of irradiation viability, induction of intracellular reactive oxygen species (ROS), apoptosis and cell death was determined. Expression of apoptotic BAX and anti-apoptotic Bcl-2 protein and their mRNA were determined by RT-PCR and Western-Blot analysis.

Results Light exposure decreased LEC viability and Bcl-2 expression and increased intracellular ROS, apoptotic cell death, and BAX expression in a time-of-irradiation-dependent manner. Phototoxic cell death and apoptosis, as well as decrease of Bcl-2 and increase in BAX expression was significantly reduced, when cells were pre-incubated with CoQ10.

Conclusion In this study CoQ10 significantly reduced light-induced LEC-damage and attenuated phototoxic effects on BAX and Bcl-2 expression. Therefore, CoQ10 supplementation might also be useful in preventing LEC death and consecutive cataract formation in vivo.

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Evaluation of the improvement of sensibility with the new IOL Master software version 5.4.3

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Purpose To evaluate the improvement of sensibility of the new IOL-Master version 5.4.3 compared with the version 4.08.

Methods 214 eyes of 115 patients were examined in a cataract preoperative evaluation making use of both IOL-Master software versions 5.4.3 and 4.08. The type and severity of the lens opacification was assessed by slitlamp biomicroscopy.

Results In 182 of the eyes examined both versions were capable to determine the AL; in 29 the version 4.01 could not determine AL whereas the version 5.4.3 was able to measure it; these cases showed posterior capsular cataract (16), dense nuclear cataract (11), posterior synechiae (1) and asteroid hyalosis (1). Finally, the AL of 3 eyes could not only be evaluated with none of the two softwares; these cases showed brunescant cataract (2) and moderate vitreous hemorrhage (1).

Conclusion The 5.4.3 software version incorporated in the new IOL-Master improves the sensibility from 85,04% to 98,59% when compared to the previous version 4.08 in the capability of measuring AL.

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Protective effect of the thioltransferase gene on in vivo UVR-300 nm induced cataract

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Purpose To determine the protective effect of the thioltransferase glutaredoxin 1 (Grx1), a critical protein-thiol repair enzyme, on in vivo UVR-induced cataract. Further, to estimate the MTD(2.3:16) for Grx1+/+ and Grx1-/- mice and to calculate the protection factor for the thioltransferase gene on UVR-induced cataract.

Methods 20 Grx1+/+ and 20 Grx1-/- mice were unilaterally exposed in vivo to UVR-300 nm for 15 min. Altogether, 5 groups of 4 animals received 0.0, 2.1, 2.9, 3.6 or 4.1 kJ/m² each. At 48 h post UVR exposure, light scattering in the exposed and contralateral not exposed lens was measured quantitatively. Macroscopic lens changes were documented with dark field illumination photography.

Results Subcapsular and cortical opacities were observed in both the Grx1+/+ and Grx1-/- mice. Forward light scattering in the lenses from the Grx1+/+ group was lower than in the lenses from the Grx1-/- group and increased with increasing radiant exposure. The MTD(2.3:16) was for the Grx1+/+ group 3.81 (CI(0.95) = [2.82;6.37]) and for the Grx1-/- group 2.99 (CI(0.95) = [2.33;4.00]) resulting in a protection factor of 1.28.

Conclusion Deficiency of the Grx1 gene makes the lens 28 % more sensitive to early onset UVR-300 nm induced cataract, indicating the important role of the Grx1 gene in the oxidation defense of the lens.

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Aspheric intraocular lenses: effect of residual spherical aberration on objective quality of vision

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Purpose To assess the impact of residual spherical aberration (SA) on objective quality of vision in pseudophakic patients.

Methods 15 patients (25 eyes) were divided into 2 groups: 13 eyes received an aspherical AcriSmart36A⁺ generating a negative SA, 12 eyes received a zero-aberration AcriSmart46LC⁺. Postoperative evaluations were conducted 6 months after a 1.8mm micro incision cataract surgery. Aberrometric measurements were done for a standardized pupil size of 5 mm in mesopic conditions using Wavescan Aberrometer⁺. Modulation Transfer Function (MTF), Strehl ratio and depth of field were measured with Objective Quality Analysis System⁺.

Results Best corrected 100% LogMar distance visual acuity (BCVA) was similar in both groups (p=0.89). 20% and 9% BCVA was increased in 36A⁺ group (p=0.009 and 0.005). Mesopic contrast sensitivity was better and total spherical aberration were significantly less with 36A⁺ IOL(SA=0.075±0.05 μm with 36A⁺ and 0.20±0.05 μm with 46LC⁺; p=0.0002). Coma and trefoil were not different between the two groups (p>0.05). 36A⁺ induced better image quality with an average MTF of 32.7±9.6 c/deg with 36A⁺ and 23.8±8.1 c/deg with 46LC⁺; p=0.0002. The 36A⁺ group had 1D less depth of focus than the 46LC⁺ group at 6 months: respectively 1.3±0.59D and 2.27±0.63D. For the whole population, depth of focus appeared to be correlated with SA (r²=0.28, p=0.02) and with trefoil (r²=0.49, p=0.0009).

Conclusion While reducing total ocular SA, aspheric IOLs improved mesopic contrast sensitivity, quality of vision with greater MTF values. Reduction of aberrations may be responsible for reduced depth of focus with 36A⁺ IOLs. However, residual trefoil aberrations may be advantageous for depth of focus and reading ability.

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Zone-I retinopathy of prematurity, progression and scheduling of treatment

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Purpose To evaluate the progression celerity and scheduling of suitable treatment time for Zone-I Retinopathy of Prematurity (ROP).

Methods Records of 36 eyes (18 infants) with Zone-I ROP, which were screened for ROP at the Neonatal Intensive Care Unit of Baskent University, Ankara, Turkey, between January 2004-March 2009, were evaluated retrospectively. Birth weight ranged between 480-1000g, gestational age ranged from 24-28 weeks. First fundus examination was performed at 29-31 weeks gestational age, and was repeated once or more per week. The first treatment was performed using laser photocoagulation and the progression criteria for laser photocoagulation treatment were:

- (1) Zone-I ROP less than stage-3 with plus disease (ETROP-type 1);
- (2) Zone-I stage 3 ROP with or without plus disease (ETROP-type 1).

Results Twenty eyes of 10 infants showed criterion (1) and 16 eyes of 8 infants criterion (2). Corneal opacity, pupillary rigidity, tunica vasculosa lentis and vitreous haze were observed until 31-33 weeks gestational age. The time period for the progression of stage-1 to stage-3 retinopathy ranged between 0.7-3.7 weeks. The mean age at the first treatment was 33 weeks (range 30-35 weeks). The mean time between the development of stage-1 retinopathy and the laser treatment was 9.8 days in mean (5-23 days), and 69.3 % of the infants were treated within 12 days after the diagnosis of stage-1 ROP. Additional treatments were performed in 7 eyes, scleral buckling+cryotherapy in 5 eyes, vitrectomy in 2 eyes. Thirty-two eyes had favorable and 4 eyes had unfavorable outcomes.

Conclusion The diagnosis of Zone-I ROP requires close-meshed follow-up and immediate treatment.

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A bilateral congenital pits of the optic nerve head. A case report

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Purpose The authors report a patient with a multiple optic nerve head pit

Methods A bilateral optic nerve head pit was found in a woman patient of 55 years old without macular retinal detachment. An 8-microm axial resolution prototype spectral domain optical coherence tomography (OCT) and stereo fundus photography were used to observe the patient. The visual acuity remained stable at 20/20.

Results The pits of the optic nerve head is a congenital anomaly of the eye and it is attributed to incomplete closure of the foetal fissure. The prevalence is 1: 10000 eyes. This depression is frequently situated in the temporal or infra-temporal region of the optic disc. There is usually only one pit per optic disc, although two or three occasionally occur. Optic pits are unilateral in 85-90% of cases. Visual acuity usually remains unaffected unless the patient develops a serous non-rhegmatogenous retinal detachment of the macula (over 60% of eyes). Visual fields characteristics of pits were found in our patient.

Conclusion High resolution OCT technology and appropriate imaging software is able to observe changes of the micro architecture of the optic nerve as this unusual pits bilateral.

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Foveal serous detachment in juvenile idiopathic arthritis(JIA)-associated uveitis

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Purpose To characterize the foveal serous detachment(FSD) in JIA-associated uveitis. To investigate the correlation with visual acuity (VA) and ocular inflammation.

Methods 9 children having FSD with JIA-associated uveitis were identified between 2005-2007. All were treated with periocular steroid injection and systemic anti-TNF α antibody. Outcome measures included VA, ocular inflammation quantified by laser flare photometry and the macular profile analyzed by OCT.

Results All patients (8 female, 1 male) had bilateral uveitis and 6 had bilateral SRD. All patients had risk factors to develop severe anterior uveitis. The mean age at the onset of uveitis and at the onset of FSD was 4.1 ± 1.1 years and 7.6 ± 2.2 years. At the onset of FSD 6 children were refractory to methotrexate and systemic corticosteroids. It had a high frequency of ocular complications: 87% posterior synechiae, 80% cataract, 60% band keratopathy and 20% glaucoma. FSD appeared isolated in 21% of eyes, it was associated with diffuse macular edema in 46% and with cystoid macular edema in 12% of cases. Before therapeutic intensification, the mean VA was 0.46 logMAR, the mean foveal thickness (FT) was 261 μ m. At 6 months follow-up VA increased to 0.22 logMAR ($p=0.017$), the reduction of flare was 41% ($p=0.003$), the mean FT was 229 μ m ($p=0.59$). At 12 months follow-up, the mean VA was 0.19 logMAR ($p=0.0029$), the mean FT was 196 μ m ($p=0.009$), only 1 eye showed persistent SRD.

Conclusion FSD is a late-stage complication of sustained and insufficiently treated anterior uveitis in JIA-associated uveitis and must be considered for the long-term visual outcome. An aggressive immunomodulatory strategy is mandatory in order to achieve strict control of ocular inflammation and improve the visual function.

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Comparison of spectral-domain and time-domain optical coherence tomography for evaluation of macular thickness and peripapillary retinal nerve fiber layer in multiple sclerosis patients

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Purpose To compare Cirrus HD Fourier-domain optical coherence (OCT) with Stratus time-domain OCT for measuring retinal and peripapillary retinal nerve fiber layer (RNFL) thickness in multiple sclerosis (MS) patients.

Methods Fifty eyes from 25 patients with MS (7 men and 18 women, aged 28 to 69 years) were prospective and consecutively studied. All of them underwent a ophthalmic examination that included assessment of visual acuity and colour vision visual field examination, Stratus OCT (Carl Zeiss Meditec, Inc.) and 3 Cirrus HD-OCT (Carl Zeiss Meditec, Inc.). Both OCT devices were used to evaluate RNFL and macular thickness. Comparison of OCTs measurements were done for the following parameters: peripapillary quadrants and RNFL clock hour sectors and the 9 macular areas corresponding to the Early Treatment Diabetic Retinopathy Study (ETDRS). Coefficients of variation (COV) using Cirrus OCT were calculated.

Results Mean RNFL average thickness were 95.70 ± 15.43 and 87.19 ± 14.52 μ m using Cirrus and Stratus OCT, respectively. RNFL measurements did not show significant differences. Mean total RT was 273.65 ± 20.17 μ m using Cirrus OCT and 200.33 ± 24.10 μ m using Stratus OCT. Retinal thickness measurements from Cirrus HD-OCT were approximately 46.91 μ m larger than those from Stratus OCT. All retinal measurements showed significant differences ($p < 0.001$). Mean COV was $3.68 \pm 2.75\%$ (ranged from 0.92 to 8.41%).

Conclusion MS patients evaluate with Cirrus and Stratus OCT show differences in their macular measurements in, but no at the RNFL. All measurements obtained using Cirrus OCT show good reproducibility in these patients.

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Rehabilitative treatment of alexia

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Purpose Homonymous hemianopia is the commonest form of acquired homonymous visual field defect; it can affect visual search and reading, producing alexia. For these patients we use different rehabilitative techniques such as eye movement-based therapies, in which the damaged visual field is more effectively sampled with compensatory or adaptative eye movements.

Methods Four left occipital stroke patients with complete right homonymous hemianopia (2 female) aged 40 to 70 years, took part. They were Spanish speaking and right-handed. All of them had complete impairment of right parafoveal vision assessed with a Humphrey perimeter. Recordings were made while the participants silently read 10 trails of single words, 10 trails each of three-and-five word arrays. For word arrays, the average fixation time per word was calculated. Single- word and text reading speed and comprehension and several cognitive functions were assessed in all patients.

Results Eye movement's therapy approaches to rehabilitation attempt to improve visual performance by the application of a regularized framework of eye movement training, with practice over 20 h of intensive training. As a group the patients had significantly shorter mean single word and text reading and comprehension after training ($p < 0.001$) and demonstrated a variety of mechanisms to account for this. Improvements were confined to the training period and maintained at follow up.

Conclusion Patients can improve visual search and reading with practice. These changes translate to improved overall reading and visual function, assessed objectively and subjectively, suggesting that they represent good training effects. The underlying mechanism may involve the adoption of compensatory eye movement strategies and these techniques all rely on mass practice.

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Thyroid eye disease and the internet

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Purpose Thyroid eye disease (TED) is influenced by modifiable risk factors yet has received little attention in terms of how the internet influences patient behaviour. We assessed the quality of information accessed by sufferers using the internet.

Methods The word 'thyroid eye disease' was subjected to an advanced keyword search. This analyses average monthly search volumes over a 12 month period using the Google search engine. The most popular search term was then inputted into Google and the first 5 pages of websites studied using a validated instrument. The 4 criteria published in the Journal of the American Medical Association (JAMA) for qualifying information from the internet were used in the analysis, yielding a possible score of between 0 and 4.

Results 100 websites were studied. All 4 criteria were satisfied by 10% of sites; 3 criteria were met by 17%; 2 criteria by 7%; 1 criterion by 20%. 46% of sites scored zero for objective quality. Superior scores were achieved by peer-reviewed journals, online abstracts, book chapters and medpedias (online medical encyclopedias). Counter-intuitively websites of professional bodies including academic institutions scored poorly. Lowest mean scores were obtained by private clinics and hospitals' websites. This is different from the quality of information available in other fields such as retinopathy of prematurity that have been studied.

Conclusion The quality of information on TED is poor and this is particularly of relevance as good patient information such as advice against smoking would improve patient outcomes. These findings are of specific relevance to how scientists and clinicians inform patients in this age where a wealth of information and misinformation is available on the internet.

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Superior oblique myokymia as a migraine motor aura

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Purpose To do an epidemiological study of the proposal that superior oblique myokymia (SOM) is a migraine motor aura and propose a pathophysiological basis for the etiology of SOM. Migraine aura is a paroxysmal spreading depression of both excitatory and inhibitory neurons in the brain. These may be sensory neurons (numbness), motor neurons (dysarthria) and inhibitory neurons (chorea). Superior oblique myokymia is bursts of very rapid, jerky contractions of a superior oblique muscle causing shaking of one eye. Interfunctioning of the cortex and basal nuclei maintains stability of eye movements. Spreading depression of the cortex would cause inhibition of the inhibitory GABAergic fibres from basal ganglia to the midbrain which could result in paroxysmal firing of the 4th nerve neurons in SOM. A finely-tuned homeostatic balance of GABAergic interneuron tone controls excitatory activation of CNS motoneurons. Small changes in GABAergic inhibition have profound effects on excitability. SOM, like auras, usually spontaneously disappears for years or permanently, which may account for the "success" of surgical treatments. The most effective treatment of SOM is gabapentin, a congener of GABA which enhances its activity.

Methods Thirty seven patients with superior oblique myokymia had detailed questioning about a history of migraine headaches and auras.

Results Every one of 37 patients with superior oblique myokymia had a history of migraine symptoms fulfilling all International Headache Society criteria. Every one had a history of migraine visual auras and many had also had sensory and dysphasic auras.

Conclusion Epidemiological correlation and a pathophysiological explanation support the proposal that superior oblique myokymia is a migraine motor aura.

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Immunohistochemical findings and proliferation markers in uveal melanomas after gamma-knife radiosurgery

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Purpose We describe immunohistochemical characteristics and tumor cell proliferation in 12 eyes with uveal melanoma enucleated after Gamma-Knife radiosurgery (GKR) with different treatment doses.

Methods Tumors were stained for the specific melanocytic markers S-100, HMB-45 and Melan A. The immunoreactive score (IRS, Remmele-score) was calculated from percentage of positive cells and staining intensity. Proliferating cells were identified with PC-10 and MIB-1 staining and counted. All markers were evaluated at the tumor apex, center, base and margins. Results were compared between eyes enucleated for tumor recurrence or treatment related side effects. Influence of treatment dose and time to enucleation were analyzed.

Results Only 1 of 12 melanomas stained positive for S-100 (median IRS 0). HMB-45 and Melan A were both positive in 11 of 12 cases (median IRS 9 and 10.5, respectively). Staining intensity was higher at the tumor apex, base and margins than in the tumor center, which frequently showed necrosis. PC-10 and MIB-1 showed proliferating cells in all melanomas, without relation to tumor recurrence (n=5), radiation dose (25-50 Gy) or time between GKR and enucleation (1-47 months). We found markedly more proliferating cells at the tumor margins.

Conclusion Proliferating cells were found even in cases with clinically stable disease. The finding of cycling cells at the tumor margins and necrosis in the tumor center might reflect the inhomogenous dose distribution used for GKR, with the tumor center receiving almost twice the dose of the surface and margins. It might also be an indicator for an intrinsically higher proliferation rate at the margins of the melanoma.

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Ophthalmologic outcome of 40 sphenoidal meningioma resections: 15 years long-term results

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Purpose To evaluate the operative results of sphenoidal meningiomas regarding resectability, recurrence and ophthalmological outcome.

Methods Retrospective study of 40 patients who underwent surgical resection of sphenoidal meningiomas followed by bony reconstruction between 1992 and 2008.

Results Mean age of 50 years and sex ratio of 37 women and 3 men. The most common preoperative sign was proptosis (90%), visual acuity (VA) and visual field deficits (55%). Periorbital tumor infiltration is a predictive factor of decreased VA in case of initial VA deficit. Optic canal invasion and resection don't influence VA recovery. Postoperatively, 10 patients showed severe permanent visual field deficits, versus 17 patients showed no visual field deficits. After surgery, proptosis improved for all patients with a preoperative mean proptosis of 5.6 +/- 3.6 mm versus 2.2 +/- 2.7 mm postoperatively (p<0.001). Lateral orbital wall resection and bony reconstruction are the two major factors which are efficient in reducing proptosis. Mean follow-up period was 7 years. Clinical tumor recurrence was observed in 10 patients (27%), radiological tumor recurrence without clinical signs was observed in 7 patients. 4 patients underwent re-operation and 3 patients were treated by radiation. Resection quality is the only predictive factor of a clinical recurrence.

Conclusion Periorbital tumor infiltration is a predictive factor of non-improvement of visual deficits (VA or visual field deficits). Peri-orbital resection leads to a better esthetic result with a better proptosis reduction. Complete and subtotal (> 90%) tumor resection gives a long-term survival free of clinical recurrence for 80% of our patients.

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IgG4-related sclerosing disease: Is there an association with orbital inflammatory pseudotumours?

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Purpose IgG4-related sclerosing disease is a systemic disease characterized histopathologically by extensive infiltration of various organs by IgG4-positive plasma cells (and T lymphocytes). Frequently associated with elevation of serum IgG4 levels, major clinical manifestations are apparent in organs in which tissues fibrosis is induced, e.g. in the pancreas. Some variants of inflammatory pseudotumour of the orbit reveal histopathologically a close relationship to multifocal fibrosclerosis. We therefore evaluated the presence of IgG4-positive plasma cells in this disease to possibly find an association with systemic IgG4-related sclerosis.

Methods We investigated 10 specimens of orbital pseudotumours that had been diagnosed by the Dep. of Pathology using HE&PAS staining. Immunohistochemical labeling was performed using antibodies against κ - and λ -chains, IgA, IgD, IgM, IgG, and IgG4 and the staining intensity was independently evaluated by MCH and HPF.

Results 3 specimens showed an increased immunoreactivity with anti-IgG4 (2/3 intensive staining, 1/3 moderate staining). In addition, in these specimens there was only a slight expression of IgA, IgD and IgM. With regard to all specimens, plasma cells secretion of λ -chains was insignificantly stronger than that of κ -chains.

Conclusion This being the first investigation of IgG4-immunostaining in ocular pseudotumours, our results indicate a possible relationship between this disease entity and IgG4-related sclerosing disease. Further investigations including IgG4 serum levels and a more detailed history regarding associated systemic disease is warranted.

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Uveal metastasis as initial manifestation of cancer

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Purpose To report clinical features of patients with uveal metastases and no known history of primary cancer at the time of ocular diagnosis.

Methods A retrospective chart review of all patients with uveal metastases and with no history of primary cancer, evaluated at University Eye Hospital in Ljubljana over a 10-year period was performed.

Results A total of 9 patients, 4 women and 5 men with uveal metastases were included. Referral diagnosis was iris tumour in 2 patients, suspected uveal melanoma in 3, choroiditis in 1 and undetermined choroidal tumour in 3 patients. None of the patients had a history of previous cancer. The diagnosis of uveal metastasis was established on the basis of medical history, clinical picture, standardised echography, fluorescein angiography and systemic evaluation. Iris and ciliary body were involved in one patient, iris in one, ciliary body and choroid in one, while choroid was the only site of metastatic involvement in 6 patients. In all patients only one eye was affected. A primary lung carcinoma was detected in 8 patients, whereas in 1 patient a primary carcinoma of the gastrointestinal tract was found.

Conclusion Lung carcinoma was the most common site of primary cancer in our patients. Patients may have no known history of primary cancer at the time of ocular diagnosis, hence a thorough subsequent systemic evaluation is mandatory.

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Orbital and periorbital cystic lesions in Indian children: A retrospective analysis from a tertiary care hospital

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Purpose Cystic lesions in orbital and periorbital area constitute a group with diverse etiopathogenesis and varied clinical and histopathologic findings. Through this study we present a review of all patients 18 years or less diagnosed histopathologically as orbital or periorbital cystic lesions, over a period of 3 years.

Methods Retrospective observational case series of histopathologically proven cysts or lesions with cystic changes, in patients 18 years or less, from Jan 2006 to Dec 2008.

Results There were a total of 97 patients, age 4 months to 18 years (mean 11.63 ± 4.48). Cysts of the surface epithelium were the most frequent lesion seen (n:50; 51.5%), of which 39 (40.2%) were dermoid cysts and 11 (11.3%) were simple epithelial cysts. Other lesions in decreasing order of frequency were: inflammatory cystic lesions - abscesses and parasitic cysts (n:16, 16.5%); neural cysts associated with ocular maldevelopment or associated with brain and meningeal tissue (n:14; 14.4%); non-cystic lesions with cystic components like lymphangioma (n:12; 12.4%); and secondary cysts - mucocoeles and lacrimal cysts (n:5; 5.2%).

Conclusion In our series cysts of the surface epithelium were the most commonly seen cystic lesion. Also noticeable is the high frequency of inflammatory lesions including parasitic and cysticercosis cysts. Cystic lesions behave clinically as benign lesions, however significant morbidity can be associated because of aesthetic considerations and vision loss owing to optic nerve compression.

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Serous retinal detachment caused by a choroidal metastasis treated with subtenon triamcinolone injection

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Purpose Ocular metastases are becoming less and less rare. Choroidal metastases are recognized as the most common intraocular malignancy. Most ocular tumors metastasize from systemic origins in breast carcinoma in females, and bronchial carcinoma in males

Methods CASE REPORTA 44 years-old woman with an history of breast adenocarcinoma three years ago, who complained of visual disturbance of the left eye. On initial examination, the left best-corrected visual acuity (BCVA) was 0,3. Left funduscopy revealed several elevated yellowish masses with peripapillary serous retinal detachment. The fluorescein retinal angiography showed a choroidal lesion highly suggestive of choroidal metastasis

Results The patient was treated with subtenon triamcinolone injection. Five months later the left BCVA was improved to 0.8. Funduscopy and optical coherence tomography confirmed reduction of edema

Conclusion CONCLUSIONSubtenon triamcinolone injection reduced subretinal edema. Optical coherence tomography (OCT) is useful in the follow-up of choroidal metastasis after treatment. However, some limitations result from the choroidal location of the metastasis.

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Comparison of mono- and bicanalicular silicone stent intubation in dacryocystorhinostomy

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Purpose Silicone stent intubation is used in DCR procedures to prevent a restenosis of the surgical ostium. However, use is not generally accepted due to concerns on cost/ effectiveness. The goal of our study was to analyze the types and rate of complications associated with monocalicular and bicanalicular silicones stent intubation and success rate of DCR procedure.

Methods A consecutive series of 114 patients who underwent 120 DCR procedures at our institution from November 2005 on has been included in the study. All DCR (transcanalicular laser DCRs) procedures were performed under general anaesthesia. One of two types of silicone intubation was inserted at each procedure: bicanalicular silicone stent (72 cases), and inferior monocalicular stent alone (38 cases). In the former a metallic clip was used to secure the stents in the nasal cavity, in the latter a double knot was applied. The intubation has been removed after 4 months.

Results We encountered the following complications: cheese-wiring of lacrimal punctum (14 cases), prolaps of intubation at the punctal side (9 cases), irritation of conjunctiva (3 cases), corneal erosion (1 case), retention of metallic clip in lacrimal sac (1 case). We statistically analyzed and compared the rate of complications for both types of intubation. Decubitus of the lacrimal punctum was less frequent with monocalicular intubation compared to bicanalicular intubation; other complications were present at similar rates.

Conclusion Complications with silicone stent intubation are not difficult to cope with and normally do not produce adverse effect on DCR success rate with the exception of early and complete prolaps of intubation, which is associated with a lesser success rate of DCR.

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Metastatic cancer presenting with ocular symptoms: a series of cases

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Purpose Choroidal metastasis is the most frequent intraocular tumour. Lung cancer in men and breast cancer in women are the most common primary sources, followed by the digestive tract. The incidence of uveal metastasis is much higher after autopsy than in clinical series so its impact is underestimated. We present a series of cases of choroidal metastasis as the initial presentation of malignant disease.

Methods We collected 5 patients in our hospital from August 2006 to April 2009 of choroidal metastasis as initial presentation of malignant disease. Primary sources, clinical and therapeutic management were analysed.

Results 3 of the 5 patients were women (60%). Ophthalmological symptoms were decreased visual acuity in 2 patients (40%) and metamorphopsia in 3 (60%). As primary source were found lung cancer in 2 cases (1 adenocarcinoma and 1 oat cell carcinoma), pancreas adenocarcinoma in 2 cases and 1 unknown origin. Only one of the patients was considered candidate to receive brachytherapy.

Conclusion Metastasis to the uvea is not a rare event due to the prolonged survival time of many cancer patients. Ophthalmologist should be aware of incidence, clinical features and management of choroidal metastasis as well as the prognosis for affected patients.

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The effect of a yellow filter on visual performance of older subjects with low macular pigment optical density

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Purpose Macular pigment (MP) filters short-wave light and it has been suggested that MP is related to visual performance. To increase the filtering properties of the eye, especially in subjects with low MP density, we can use a yellow filter that absorbs the amount of blue light striking the retina. This study was performed to relate MP density levels to variations in the mesopic visual function and light scattering provoked by the yellow filter in old subjects.

Methods Measurements were obtained from 88 healthy old subjects aged 70 ± 6.3 years. The optical density of the MP was estimated at the fovea using the Metropsis Test (Cambridge Research System). Two groups were established in function of the MP level: $MP \leq 0.2$ (41 subjects) and $MP > 0.2$ (47 subjects). Visual acuity, contrast sensitivity and scattered light were measured in the right eye with and without a yellow filter under mesopic luminance conditions. Visual acuity was measured using high-contrast (96%) and low-contrast (5%) logMAR letter charts. Contrast sensitivity was determined using the Pelli-Robson chart. Scattered light on the retina was measured using the C-Quant straylight meter (Oculus).

Results Mean foveal MP optical density was 0.22 density units (SD 0.13; range 0.0 to 0.64). Mean mesopic high-contrast and low-contrast visual acuity, contrast sensitivity and log scatter recorded with a yellow filter did not show any significant improvement or worsening compared with no filter condition in both MP groups.

Conclusion In healthy old eyes with low macular pigment optical density mesopic visual function and intraocular light scattering did not improve from the use of a yellow filter.

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Optical filters influence on pupil size according to their material and spectral characteristics

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Purpose To evaluate pupil size variations induced by optical filter by using filters with different materials and spectral characteristics, focussing on UV and blue light transmission properties of the filters

Methods 220 pupil measurements (20 eyes, 20-55 years old) were made using the pupillometer Procyon P2000SA at high Mesopic level (4.0lux). 10 filters of varying transmittance (visible, 4%-65%, 5 filters UV-transmitting and 5 filters UV-absorbing) were used in a randomised way. 5 filters (UV-transmitting) were dye-coated polyester films with a substrate of PET (polyethylene terephthalate) and the another 5 filters (UV-absorbing) were Ophthalmic CR39 lenses with a tinted coating in order to get the same absorbing characteristics than the PET filters. Measurements in the absence of a filter served as control

Results Significant results on pupil size variation were obtained with lower UV and blue light transmittance filters (F14, F15 and F16; $p=0.00$) for both materials, PET and CR39 (Mydriasis obtained: F14R=13.99%; F15R=17.57%; F16R=22.90%; F14E=23.95%; F15E=22.69%; F16E=29.87%). Also significant differences in mydriasis-induced by the filter were obtained when materials and blue-light transmission properties were compared for filters 14 and 16 (PET vs. CR39: F14 ($p=0.006$), F16 ($p=0.0167$))

Conclusion Filters with similar visible but different UV and/or blue-light transmission properties significantly produce variations in pupil size at high Mesopic condition (4.0lux). Differences obtained in mydriasis-induced by the filter depend not only on the material of the filter, but also the UV and/or blue-light transmitted by the filter

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How does normal crowding affect visual acuity?

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Purpose Clinical tests of visual acuity (VA) that employ multiple, neighbouring optotypes assume that visual 'crowding' at the fovea is negligible. Findings from recent studies suggest that crowding effects can affect high contrast acuity thresholds at the fovea. The absence of data to describe the distribution of crowding effects within 'normal' vision makes it difficult to establish when a measured reduction in VA (with crowding) can no longer be considered to be within the normal range and is therefore indicative of abnormal development or pathology. The aim of this study was to quantify the effects of crowding on VA in the normal population.

Methods We measured acuity thresholds, with and without crowding, in central vision (i.e. at the fovea and at +/-1 degree, +/-1.5 degrees, and +/-2 degrees) in 80 normal subjects with the age range of 29.3 +/-10.7 years. The stimulus optotype was a Landolt ring of 100% luminance contrast presented either in isolation or together with four surrounding rings at a distance equal to 1.5 times the diameter of the stimulus, these parameters were selected on the basis of preliminary studies varying the spacing and the number of distracters.

Results The threshold stimulus size for correct discrimination of gap orientation increased almost linearly with eccentricity, the presence of distracter rings caused a significant reduction in VA.

Conclusion The statistical distribution of the differences between the two measures of VA provides the data needed to define the effects of crowding in "normal" vision. The template extracted from these data is clinically useful to identify those subjects that show abnormal sensitivity to crowding, i.e. amblyopia or early degenerative conditions/disease.

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Optico-anatomical changes of prematurity children eyes



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Purpose The aim of our work was to determine changes in optical-anatomical elements of myopic eyes of full-term and premature children during accommodation by means of precise ultrasonic biometry.

Methods The study was made on healthy full-term children's eyes with emmetropic refraction (group, $n = 20$); full-term 1st degree myopic children's eyes with refraction from -1.0 D to -3.0 D (group 2, $n = 16$), and premature children's myopic eyes with refraction -1.0 D to -3.0 D (group 3, $n = 12$). The age of children ranged from 6 to 15 years. Gestation age in the pre-term group ranged from 25 to 34 weeks. All were seen in the clinic for the risk of developing retinopathy of prematurity.

Results Ultrasonic biometry evaluates optical-anatomical parameters in premature children's myopic eyes. Axial length was longer than in healthy and full-term myopic children's eyes (mean, 24.09 ± 0.69 mm) and lens thickness was bigger (mean 3.35 ± 0.14 mm). In the healthy children's group: axial length was 23.49 ± 0.48 mm and lens thickness 3.00 ± 0.07 mm, and in the full-term myopic children's group the axial length was 23.79 ± 0.59 mm and lens thickness 3.24 ± 0.14 mm.

Conclusion In the group of premature children changes in the optical-anatomical elements parameters of myopic eyes were more pronounced than in the group of full-term children, and it could lead to higher myopia development.

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Wavefront map analysis of single vision and progressive ophthalmic lenses

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Purpose To evaluate and compare wavefront aberration maps in single-vision (SVL) and progressive power lenses (PPL) of different materials and designs.

Methods The monochromatic wavefront aberrations of SVL and PPL were evaluated using a Shack-Hartmann wavefront sensor. The lenses were categorized according to their material, power, design and refractive index. Measurements were taken along vertical and horizontal axes starting from the lens optical center and moving towards its periphery in 3mm steps. Artificial pupils of 3mm and 6mm diameter were used to simulate photopic / mesopic viewing conditions. For each point the average of three measurements was analysed. Zernike expansion coefficients up to 4th order, defocus sphere, astigmatism and paraxial curvature matching were calculated using custom-made scripts in Matlab computational software.

Results In all cases the magnitude of aberrations was higher when a 6mm viewing pupil was used. The most prominent aberrations were the second order astigmatic components, especially in PPLs and positive SVLs. Spherical aberration and third order aberrations (coma and trefoil) were more pronounced in specific designs. Finally, the magnitude and the pattern of aberrations are affected by lens material, power and refractive index.

Conclusion Wavefront aberration data offers, in addition to the common power mapping, the evaluation of the higher order aberrations profiles of the lens, which may compromise visual performance. This allows a more accurate assessment of the visual enhancement provided by modern PPLs with advanced designs.

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The effect of ocular higher order aberrations on contrast sensitivity.

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Purpose The objective of the study was to evaluate the impact of ocular higher order aberrations on the contrast sensitivity function(CSF).

Methods Twenty-one subjects (age:25.4±5.0) volunteered to participate in the study.Best spectacle-corrected contrast sensitivity of the dilated dominant eye was evaluated using vertical gratings (Gabor patches with a sd of 1.2deg at 2m distance) displayed on a Sony F520 CRT monitor by means of a VSG2/5 stimulus generator card. Average screen luminance was 30cd/m2.Contrast sensitivity was assessed for 6 spatial frequencies(1,2,4,8,12 and 16c/deg) and for artificial pupils of 3 and 6mm diameter. Retinal illuminance was equalised in both conditions by using an NDfilter.Threshold was determined using a binary-search staircase with a contrast resolution of 1dB.Ocular aberrations were recorded at 6mm pupil with a COAS wavefront sensor(Wavefront Sciences Ltd).Objective image quality metrics,e.g Modulation Transfer Function(MTF) and Root Mean Square(RMS)wavefront error,were calculated using custom-written scripts in Matlab.

Results CSF and MTF were fitted using 3rd order polynomials.The area calculated by the integral of the fitted functions was used as a measure of performance.CSF was found depressed for 6 compared to 3mm pupils,as was the case for the MTF.Moreover,the decrease in performance at 6mm was always more pronounced for the MTF than the CSF.No significant correlation was found between the CSFarea and the respective MTFarea or any other image quality metrics.

Conclusion Ocular higher order aberrations adversely affect contrast sensitivity,particularly at high spatial frequencies.However,objective measures of higher order aberrations,such as the MTF and the RMS error cannot simulate their impact on the CSF.

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Anterior segment biometrics – investigation of hyperopic eyes with Orbscan

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Purpose Assessment of the anterior segment by Orbscan in hyperopic eyes.

Methods 30 normal right eyes (10 younger hyperopes, 10 older hyperopes, 10 younger emmetropes) were assessed with Orbscan II. Anterior chamber depth (ACD), horizontal corneal diameter (HCD), anterior (AK) and posterior corneal radius (PK), central (CCT) and peripheral corneal thickness (PCT, 4mm from centre) were analysed in the younger (≤ 45 years of age) and older hyperopic subjects (> 45 years). The data from the younger hyperopic group were also compared with the age-matched emmetropic subjects.

Results All data were normally distributed ($p \geq 0.403$). For the 20 hyperopic subjects, the mean refractive error was +2.33 D (SD+/- 1.85 D), the mean ACD was 2.59 +/- 0.35 mm, with a mean HCD of 11.76 +/- 0.33 mm. The mean ACD was less in young hyperopes than in emmetropes (2.72 mm vs 2.91 mm). ACD was a further 10 % shallower in older hyperopes when compared to younger hyperopes, with the peripheral cornea also being slightly thinner (by about 4 %). No substantial differences between the younger and the older hyperopes were noted in HCD, PK, and CCT ($p \geq 0.094$, independent samples t-test), but younger hyperopes had on average a larger AK than older subjects, the difference being only just significant ($p = 0.02$).

Conclusion These pilot studies indicate that most anterior segment biometrics are not substantially different between younger and older subjects with hyperopia. However, younger subjects had a slightly flatter anterior corneal radius and a thicker peripheral cornea, along with the expected (on average) lower ACD in hyperopic eyes and older subjects. Further studies are needed for refractive surgery procedures in older hyperopic patients.

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Validation of Modified Grating Test as a visual acuity test in low vision patients with retinitis pigmentosa

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Purpose The Modified Grating Test is a newly designed test for visual acuity (VA) measurement in patients with retinitis pigmentosa (RP) with low vision. Freiburg Visual Acuity Test (FrACT) is a validated test for low vision, but no patients with RP were tested. In our study we tested RP patients investigating the consistency of the Modified Grating Test by comparing the results with those of the FrACT and the ETDRS charts.

Methods 16 eyes of 8 patients were tested and divided into 3 groups depending on their VA. Group 1 had a VA of hand motion (HM, n= 6), group 2 counting fingers (CF, n=6) and group 3 better than CF (n=4). The Modified Grating Test consists of a circle with black and white bars of the same width in a decreasing logarithmic order covering the range from 2.7 to 0.3 logMAR. The FrACT is a computerized test with Landolt C in different directions and in decreasing order. ETDRS charts were used at a distance of 0.5m. Each test was performed 4-5 times. The data were normally distributed. To compare the values we used the paired samples t-test.

Results The mean VA in group 1 as measured by the Modified Grating Test was 1.3 logMAR and by FrACT 1.21 logMAR ($p=0.21$). In the group 2 the patients reached a mean VA of 0.7 logMAR with the Modified Grating Test and 0.6 logMAR with FrACT. 0.68 logMAR was measured with ETDRS charts ($p=0.13$).

Conclusion Our preliminary data suggest that the Modified Grating Test is a suitable test for the low vision range and might be beneficial for the follow up in patients undergoing a retinal prosthesis implantation.

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The AGREE instrument applied to visual health: an assessment of the health protocols used in Spain to measure the effects exposure to VDT

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Purpose Developing an instrument for appraising health protocols and testing its application through the assessment of a specific clinical protocol applied in Spain to protect the visual health of workers using computers.

Methods In the development of the appraisal tool, the Spanish version of the AGREE instrument was taken as the starting point and modifications were then introduced.For the assessment of the health protocol a Delphi technique and the nominal group were used. A group of 9 experts carried out the individual anonymous assessment. The level of agreement between experts for responses to each of the questions was defined, as was the number who had chosen the same option for closed responses.

Results The appraisal tool comprised 13 questions organised into 5 domains, and one final question aimed at eliciting a global evaluation of the protocol.Regarding the agreement between experts, this exceeded 50% for 10 of the 14 questions. Responses most frequently chosen by the assessors for all of the questions were the least favourable to the protocol indicating the protocol significantly failed to fulfil the conditions addressed in the majority of the questions considered.

Conclusion Application of this appraisal tool has enabled a good degree of agreement to be reached for experts' responses when assessing aspects related to the clinical protocol for visual health of workers using computers demonstrating its applicability and capacity for obtaining conclusive widely endorsed assessments.

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Visual symptomatology associated with workers using VDT: a review in the last decade

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Purpose Reviewing epidemiological studies concerning the visual symptomatology associated with workers using VDT.

Methods A literature search was carried out of articles published 1999-2008 in the databases: MEDLINE, Biological Abstracts, INSPEC, PsycINFO, FSTA, CINAHL, CC Search, FRANCIS and The Cochrane Library. Papers published in Spanish, English, French and Italian was reviewed. The search procedure combined the MeSH descriptors asthenopia, computer terminals and work in addition to the open expressions visual fatigue and computer vision syndrome. Various search equations were established, using Boolean operators, truncation and filters. Inclusion and exclusion criteria related to the target population, the research aims, and type of publication was defined. Research quality was assessed. A data collection protocol was developed in order to analyse the contents of the publications selected.

Results Sixteen original cross-sectional articles were included. In 9 of these the term asthenopia was used to refer to this symptomatology. A high level of repetition could be observed in the selection of symptoms but there was no agreement on definition of scores or global criteria for establishing whether a worker was suffering from asthenopia or not.

Conclusion It is necessary to develop an instrument capable of assessing occupational asthenopia associated with VDT which includes all of the ocular and visual symptoms found during the review. However, it is also necessary to carry out research to analyse the association between symptoms and exposure, thus justifying the greater importance given to some symptoms rather than others when deciding whether asthenopia is present or absent in these workers.

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The damaging effect of various intensities of light on retinal function in pigmented rats.

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Purpose To investigate the functional retinal damage following the short term (6h) light exposure in pigmented rats.

Methods One group of dark adapted Brown-Norway rats was exposed for 6 h to 10 klux without mydriasis. Five groups of rats were exposed to different intensities of light (10, 5, 3.4, 2.7 and 1.7 klux) for 6 h with pupil dilatation. Electroretinography (ERG) was recorded prior to, and at 1st and 7th day after light damage (LD). ERG protocol enabled quantification of scotopic sensitivity parameters (V_{max} -saturated b-wave amplitude and k- luminance to reach $V_{max}/2$), photopic cone response and oscillatory potentials (OPs).

Results The exposure to 10 klux without mydriasis did not result in significant retinal injury. In groups subjected to mydriasis, the light of 1.7 klux resulted in nonsignificant (ns) decrease of ERG response. However, the severe loss of retinal function was reported after exposure to higher intensities. The value of V_{max} at 1st day after LD was reduced respectively to $90 \pm 25\%$ (ns), $19 \pm 5\%$, $7 \pm 4\%$, $4 \pm 1\%$ and $3 \pm 0.3\%$ of baseline value ($p < 0.05$). No significant improvement was recorded during the following 7 days. The parallel decrease was observed in scotopic mixed (rod+cone) a- and b-wave amplitude, in photopic response and in OPs. The scotopic threshold luminance increased gradually together with the damaging light intensity, while the value of parameter k was only narrowly altered ($p > 0.05$) after LD in all animal groups.

Conclusion Our results show, that a few hours' illumination with the light of even moderate intensity may result in severe functional retinal impairment in pigmented rats. Presented model may be useful to study the cell degenerations in pigmented retinas.

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Electroretinogram alterations in a monozygotic twin sisters discordant retinitis pigmentosa

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Purpose We describe the electroretinographic alteration in an interesting and unusual case of a twin sisters discordant Retinitis Pigmentosa (RP).

Methods A 30-year-old woman complained of night blindness. She and her twin sister, underwent complete ophthalmological exams including funduscopy, retinography and electroretinogram at the Clinical Hospital of the University of São Paulo.

Results The scotopic full-field electroretinogram was non detectable and not significantly different from noise. The photopic and flicker responses were present, but abnormal. The multifocal electroretinogram (mfERG) showed a residual central cone response. The scotopic and photopic full-field electroretinogram and mf-ERG of the sister were normal.

Conclusion The discordant RP is very rare and the electrophysiological tests are important to confirm diagnosis. We believe that RP can manifest in incomplete penetrance or in different phenotypes.

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Analysis of multifocal electroretinography implicit times in the prediction of macular hole formation

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Purpose Our aim was to investigate the predictive role of multifocal electroretinography (mfERG) by analyzing the implicit time changes in the fellow eye of patients with unilateral macular hole.

Methods 80 eyes of 40 patients with unilateral macular hole were examined by mfERG (RetiScan, Roland Consult, Germany). Ring ratios of the multifocal electroretinograms were calculated by correlating the central rings' implicit time with that of the peripheral ones (T1/T2, T1/T3, T1/T4 and T1/T5). After vitreoretinal surgery, during the follow-up period of 24 months, in 13 of 40 cases macular hole formation was detected in the previously intact fellow eye by optical coherence tomography. Patients were divided into two groups according to this. The implicit time ring ratios of the two groups were compared.

Results Surprisingly, although the implicit times showed no significant difference in the two groups, the ratios T1/T2, T1/T3, T1/T4 and T1/T5 were significantly lower in the eyes that later developed macular hole (1.08, 1.12, 1.14, 1.16) than those which remained intact (1.01, 1.03, 1.05, 1.07, respectively; $p < 0.05$).

Conclusion By analyzing the mfERG implicit time, using ring ratios seems to be a more sensitive method in predicting macular hole formation in the fellow eye, than analyzing the implicit times alone. Lower values might forecast propensity for subsequent macular holes. These patients may require stricter follow-up.

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Morphofunctional changes of macular area in diabetic macular edema

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Purpose To investigate the electrogenesis in the L- and S-cone system of the retina and their role in diagnosis of focal and diffuse macular edema (ME) in diabetic retinopathy (DR).

Methods 30 patients with diabetes mellitus type II were tested. All patients had ME: focal – 24 eyes, diffuse – 21. ISCEV Standard electroretinography (ERG) tests and additional chromatic macular ERG (M-ERG) were used. Psychophysical test was presented by screening a topography of contrast and color sensitivity of the cone system. OCT was used to confirm macular edema that was found ophthalmoscopically.

Results In focal and diffuse ME a similar decrease of amplitude (M=6 mv; norm M=18 mv) and prolonged latency of b-wave (M=58 ms; norm M=54 ms) to red stimuli were registered. M-ERG to blue stimuli was subnormal in all cases: b-wave amplitude in focal ME was M=23 mv, in diffuse M=26 mv (norm M=62 mv). The b-wave latency of M-ERG to blue (norm M=62 ms) was prolonged mostly in focal (M=80 ms) than in diffuse ME (M=76 ms). Campimetry for color sensitivity to red and blue stimuli revealed significant changes in all patients, dark channels affected earlier than light.

Conclusion In focal and diffuse ME similar changes of chromatic M-ERG to red and blue stimuli are an evidence of cone function suppression. Prolonged b-wave latency is due to excitation delay from photoreceptors to ganglion cells as a result of neuronal damage. Decreased sensitivity of dark and light channels with dominant localization of pathological process in macular area were found. The functionally revealed changes in focal ME may reflect the extensiveness and prevalence of retinal damage unlike the clinical picture.

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Development of a software for the exchange of electrophysiological data of vision with PACS systems based on DICOM

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Purpose Digital imaging and communications in medicine (DICOM) is widely acknowledged as standard in medical informatics. It provides a communication protocol for interchange of diagnostic and therapeutic information, images, and any associated data. Medical devices with DICOM interfaces can be connected to share data. DICOM evolves continuously in supporting new modalities and services and now allows also the interchange of waveforms. In electrophysiology of vision however, storage and exchange of data is mostly based on proprietary protocols of device vendors. We developed a software for vendor neutral storage of electrophysiological data based on DICOM.

Methods DICOM follows the object-oriented paradigm and defines information object definitions (IODs), describing the type of data dealt with (e. g. CT image) as well as service classes (SC) specifying valid operations for specific IODs (e. g. store). SCs and IODs form so called service object pairs (SOP). In this work, the IOD for waveform data was extended using private attributes as designated by the DICOM standard. A service class user (SCU) for the connection to a picture archiving and communication system (PACS) was developed. The software is based on the PixelMed Java DICOM Toolkit. Testing was done using dcm4chee as PACS.

Results The software is integrated in Tuebingen ERG Explorer, allowing for submission of electrophysiological recordings to a PACS. It is used as a prototype at the University Eye Hospital Tuebingen.

Conclusion We have introduced a IOD for storage of electrophysiological data of vision as a first step of integration into a distributed eHealth network, leveraging existing DICOM infrastructure like PACS.



■ 401 / 4356

Topical levofloxacin 1.5% Is effective in reducing levofloxacin-resistant MRSA and FQ-resistant pseudomonas aeruginosa in keratitis models

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Purpose We compared topical levofloxacin 1.5% (LEV) to standard therapies in reducing levofloxacin-resistant *Staphylococcus aureus* (MRSA) and FQ-resistant *Pseudomonas aeruginosa* (PA) in keratitis models.

Methods Both corneas of 32 NZW rabbits were intrastromally injected with 1000 CFU of MRSA (LV MIC=32 µg/ml; van MIC=2 µg/ml). After 4 hours (onset), 8 rabbits were sacrificed and the corneas were homogenized for colony counts. 24 rabbits were divided into 3 topical treatment groups: 1) LEV, 2) vancomycin 5%, and 3) saline. Treatment consisted of drops every 15 minutes/5 hours (21 drops). One hour after treatment, the rabbits were sacrificed and the corneas were homogenized for colony counts. Similar to MRSA, 32 NZW rabbits were intrastromally injected with 1000 CFU of PA (LV MIC=32 µg/ml, tob=0.75 µg/ml). After 16 hours (onset), 8 rabbits were sacrificed and the corneas were homogenized for colony counts. 24 rabbits were divided into 3 topical treatment groups: 1) LEV, 2) tobramycin 1.4%, and 3) saline. Treatment consisted of drops every 15 minutes/1 hour and every 30 minutes/7 hours (19 drops). One hour after treatment, the rabbits were sacrificed and the corneas were homogenized for colony counts. The CFU data were analyzed non-parametrically.

Results LEV significantly reduced more MRSA than vancomycin 5%, and both significantly reduced more MRSA than the onset and saline control (p<0.05). LEV was as effective as tobramycin 1.4% in reducing PA, and both significantly reduced more PA than the onset and saline control (p<0.05).

Conclusion LEV was more effective for reducing MRSA and as effective for reducing PA as standard therapies in rabbit keratitis models.

Commercial interest

■ 403 / 4358

In vitro antimicrobial effect of vitreous endotamponading substances

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Purpose To demonstrate the in vitro bactericidal, bacteriostatic or inert role of endotamponading substances.

Methods Clinical isolates of *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Clostridium* sp were cultured on blood agar plates in sulphur hexafluoride (SF6) and perfluoropropane (C3F8) atmospheres and under silicone oil (SO) and liquid perfluorocarbon (LPFC). The same germs were cultured under aerobic and anaerobic atmospheres as controls.

Results SO, SF6 and C3F8 did not significantly inhibit the growth of anaerobic bacteria but markedly reduced the growth of aerobic germs, as compared with aerobic conditions. The growth of the bacteria under SO and SF6 and C3F8 was very similar to that observed under anaerobic conditions. LPFC did not affect the growth of aerobic or anaerobic bacteria.

Conclusion Endotamponading gases and liquids seem to improve the visual and anatomical outcome of infected eyes and may limit the proliferation of aerobic germs in vitro. It is not known to what extent the antimicrobial effect of these substances would appear in vivo since oxygen diffusion from the blood could allow the growth of aerobic germs.

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TNFα suppresses IFNγ-induced MHC Class II expression in retinal pigmented epithelial cells by down regulating Class II trans-activator mRNA

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Purpose The expression of MHC class II molecules induced by IFNγ on RPE cells plays a crucial role in the development of autoimmune uveitis. As TNFα is commonly coexpressed with IFNγ in this disease, we have investigated the effects of TNFα on the IFNγ mediated MHC II induction in RPE cells.

Methods ARPE-19 were cultured and stimulated with TNFα, IFNγ, IL-1β and different combination of these cytokines. After cytokine treatment, we have analysed the expression of MHCII and ICAM-1 by flow cytometry. IDO induction was studied by Western blotting. The activation and expression of two proteins involved in IFN pathway: IRF1 and STAT was analysed by Western blot. We have also monitored the effects of TNFα and IFNγ on the expression of CIITA by quantitative RT-PCR.

Results TNFα, but not IL-1, inhibits IFNγ induced MHC class II expression on the surface of ARPE cells. We did not observed an inhibitory effect of TNFα on the expression of ICAM and IDO induced by IFNγ. Similarly, STAT1 phosphorylation and IRF1 induction obtained after IFNγ treatment were not affected by TNFα. On the contrary, we found that TNFα suppresses IFNγ induced CIITA mRNA accumulation.

Conclusion TNFα suppresses IFNγ induced CIITA mRNA accumulation and MHC II expression in human retinal pigment cells.

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An epidemiologic analysis of staphylococcus aureus-associated keratitis in Boston

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Purpose *S. aureus* is a normal commensal of the human skin and nasopharynx. It is therefore of interest to determine whether *S. aureus* keratitis is caused by a subset of these organisms. In this study, the phenotypic and genotypic characteristics of *S. aureus* keratitis isolates were analyzed.

Methods All *S. aureus* clinical isolates were prospectively collected over a 24 month period at the MEEI (2006-2008). The diagnosis of clinical keratitis and associated risk factors was by medical record review. Keratitis-associated *S. aureus* strains were assessed for:

1. antibiotic susceptibility,
2. biofilm robustness by gentian violet staining using an in vitro microtiter plate assay, and
3. genetic lineage by multi-locus sequence typing (MLST).

Results 26 cases of keratitis were identified from the 600 *S. aureus* clinical isolates. Risk factors associated with *S. aureus* keratitis included trauma, prior surgery, soft contact lens wear, and the presence of a foreign body. Ocular surface disease does not appear to be an independent risk factor. All 26 isolates were tetracycline- and trimethoprim-sulfamethoxazole- sensitive. All the MRSA strains were found to be ciprofloxacin-resistant (10/26). Nearly one-half of all the *S. aureus* keratitis-associated isolates were caused by a single clone, ST5. Both methicillin sensitive and resistant *S. aureus* strains were represented within ST5.

Conclusion These results suggest that there may be specific *S. aureus* lineages which possess phenotypic and genotypic characteristics that enable *S. aureus* to more effectively cause sight-threatening keratitis. Future work will examine their virulence traits and a comparison to commensal *S. aureus* strains.

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Comparative assessment of *S. aureus* microbial biofilm inhibition by an N-alkyl-polyethyleneimine covalently attached to PMMA or titanium in the Boston Keratoprosthesis

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Purpose Biofilms are matrix-associated microbial communities adherent to either biological surfaces or abiotic surfaces. They account for the majority of device-associated infections. Our goal herein is to minimize bacterial adherence and biofilm formation by comparative analysis of new polycations bound to bio-prosthetic ocular-associated materials, poly (methyl methacrylate) (PMMA) or titanium, using the Boston KPro as a model system.

Methods Using *S.aureus* ocular-associated clinical isolates, a quantitative assessment of microbial biofilm formation by linear N,N-dodecylmethyl-polyethyleneimine (DMPEI) (217 kDa) covalently bound to PMMA or titanium compared to the parent PMMA or Ti, respectively, has been performed using confocal laser scanning and electron microscopies. In addition, DMPEI-bound materials have been screened for corneal toxicity in both cell tissue culture and rodent models, and as compared to the original materials.

Results A marked inhibitory effect in *S.aureus* biofilm formation on DMPEI-derivatized materials compared to the parent PMMA (3-4 fold) and Ti (2-fold), without conferring additional epithelial cell cytotoxicity in vitro, has been observed. Furthermore, we have found no additional tissue reactivity, and possibly even a protective effect, with DMPEI-derivatized materials in vivo.

Conclusion We found that covalent derivatization with DMPEI of PMMA and Ti greatly reduces *S. aureus* biofilm formation in vitro compared to the parent materials. There was no additional cytotoxicity seen both in vitro and in vivo. Future studies will evaluate DMPEI-derivatized materials for in vivo antimicrobial efficacy.

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Implantation of intraocular lens in children with uveitis: long term visual outcome and prognosis

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Purpose To evaluate long term visual acuity, intra ocular inflammation and late complications for children with chronic uveitis who underwent cataract surgery with posterior chamber intra ocular lens (IOL) implantation.

Methods Retrospective, monocentric study of children under 16 with chronic uveitis and who underwent cataract surgery between January 1996 and December 2001. Surgical protocol included: phacoaspiration with posterior capsulorhexis, anterior vitrectomy and IOL implantation. Visual acuity, intra ocular pressure, slight lamp examination, laser flare photometry and anti-inflammatory treatment were analyzed at different time points.

Results 17 eyes of 12 children (8 boys, 4 girls) were included. Uveitis was bilateral in 6 cases. The mean age at surgery was 10.5 years (ranged from 4 to 15) and the mean follow up time was 8.9 years (from 7 to 13). Diagnosis of uveitis was idiopathic juvenile arthritis in 8 cases (64.7%), sarcoidosis in 1 case and finally no etiology was identified in 3 cases (29.4%). Seven years after surgery, the mean visual acuity was 0.33 logMAR and 82% of children had still an immunomodulatory treatment. Late complications were: glaucoma (11.7%), Elschnig pearls (11.7%), giant cells on IOL (35%) and CME (6%) of cases.

Conclusion IOL implantation in children with chronic uveitis prevents amblyopia and allows rapid visual recovery. Complete surgical procedure in this group of children has interesting results on visual acuity and rehabilitation. Strict and long-term control of intraocular inflammation is mandatory in order to prevent major complications and blindness. Implantation should not be proposed in young children with JIA-associated uveitis.

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Three years experience of Quantiferon®Gold testing

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Purpose To give an overview of the results for Quantiferon® Gold (QFT) testing on uveitis patients in an interdisciplinary setting for a period of three years.

Methods Database search of all the patients tested for tuberculosis (Tb) with a full blood gamma interferon release assay, QFT, the result of the testing and analysis of uveitis subtypes involved.

Results From March 2006 until April 2009 294 patients have been tested, overall 62 (21.8%) were positive and 220 (74.8%) negative (in 2 the result was unclear). 101 patients were male, 193 female, age mean was 49 (13 – 86) years in the whole cohort and 50 (22–80) years in the QFT+ group. Looking at anatomic localization they were distributed (in brackets % of QFT+ tests) as anterior n=46 (9%), intermediate n=68 (26%), posterior n=87 (26%), and pan n=38 (32%). In 26 QFT+ patients we presumed a diagnosis of Tb due to other clinical findings. In 14 patients a final diagnosis of Tb associated uveitis was postulated and treated with full therapy following WHO recommendations.

Conclusion QFT testing gives surprisingly high numbers of positives in uveitis patients, especially in the subgroup of intermediate, posterior and panuveitis. This is not sufficiently explained by immigrant status of the patients. The frequency of positives is a lot higher than in other cohorts as healthy hospital personnel (4.8%, own data) or inflammatory arthritis patients (9.8%). This opens important questions regarding treatment implications. Martin J, et al. Ann Rheum Dis. 2009 Jan 30.

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Aqueous humor heparin-binding epidermal growth factor-like levels in patients with acute anterior uveitis.

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Purpose Heparin-binding epidermal growth factor-like growth factor (HB-EGF) is a potent antiapoptotic protein that enables cells and tissues exposed to different apoptotic stimuli to survive hypoxic, oxidative, and inflammatory stresses. The aim of this study was to evaluate the levels of HBEGF (HBEGF) in the aqueous humor in eyes with acute anterior uveitis (AAU).

Methods A prospective, comparative control study. Aqueous humor was collected from 16 eyes of 16 patients with AAU. The level of HBEGF was determined with a commercially available enzyme-linked immunosorbent assay kit. The control group comprised 16 aqueous samples from 16 patients about to undergo cataract surgery and without any other ocular or systemic diseases.

Results The concentration of HBEGF in aqueous humor was markedly higher in patients with UUA than in control subjects (Mann-Whitney U test, $P < 0.001$). The level of HBEGF was 45.18 ± 54.58 (mean \pm SD) pg/ml in eyes with UUA and 8.15 ± 5.71 pg/ml in the eyes of the control group.

Conclusion The aqueous humor HBEGF level is increased in eyes with AAU. These results imply that HBEGF is associated with the regulation of inflammation in patients with AAU

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Aqueous humor keratinocyte growth factor levels in patients with acute anterior uveitis.

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Purpose Keratinocyte growth factor (KGF) is produced by cells of mesenchymal origin and has an important role in repairing tissues and possesses anti-inflammatory properties. The aim of this study was to evaluate the levels of KGF in the aqueous humor in eyes with acute anterior uveitis (AAU).

Methods A prospective, comparative control study. Aqueous humor was collected from 16 eyes of 16 patients with AAU. The level of KGF was determined with a commercially available enzyme-linked immunosorbent assay kit. The control group comprised 16 aqueous samples from 16 patients about to undergo cataract surgery and without any other ocular or systemic diseases.

Results The concentration of KGF in aqueous humor was markedly higher in patients with UUA than in control subjects (Mann-Whitney U test, $P < 0.001$). The level of KGF was 38.06 ± 37.46 (mean \pm SD) pg/ml in eyes with UUA and 3.50 ± 1.3 pg/ml in the eyes of the control group.

Conclusion The aqueous humor KGF level is increased in eyes with AAU. These results imply that KGF is associated with the regulation of inflammation in patients with AAU.

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Inhibition of viral replication in vitro by antiviral-treated amniotic membrane

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Purpose To investigate if amniotic membrane soaked with antivirals could inhibit viral growth in vitro

Methods Amniotic membrane fragments were incubated with antiviral solutions of acyclovir and trifluridine. After having inoculated vero cells with a viral suspension of HSV1 and added with the AM fragments we tested them for the antiviral activity by recording the cytopathic effect and DNA assessment by Quantitative-PCR.

Results AM soaked in ACV 50 mg/ml was capable to inhibit the development of HSV viral growth in cell cultures. Non-treated AM did not significantly affect viral replication.

Conclusion Our preliminary in vitro data show that amniotic membrane can absorb the antivirals

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A case of intraocular dirofilaria in a dog: alarm bell for human beings?

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Purpose The genus *Dirofilaria* includes various species, in particular immitis and repens, that are natural parasites of dogs and other domestic and wild mammals. From those natural hosts microfilariae circulating in blood may accidentally be transmitted by mosquitoes to man. These microfilariae can be localized in the human beings' periorbital or subconjunctival tissues or in the intraocular region. This case report observed an increase of the ocular filariasis in dogs and it aims to put emphasis on the possible transmission of this parasite from animals to the human eye.

Methods A 2-year-old male, mixed breed living in the North of Italy, in the Emilia Romagna area, was presented to the author having pain to the right eye. Ocular examination showed miosis, corneal edema and low intraocular pressure. Slit lamp examination revealed a lively motile worm in the anterior chamber.

Results It was not possible both to remove the parasite and to identify its species. At the same time no parasite was identified in the peripheral blood.

Conclusion The rise of weather temperatures of these last years caused an increase in the occurrence of "tropical disease". This particular case shows many similarities to the human parasite because the dog had no microfilariae in the blood. Many studies pointed out a remarkable incidence of microfilariae in the dogs' eyes living in the Mediterranean area; this remark must be taken into serious consideration because it can be considered as an emerging zoonosis and as an alarm bell for human beings.

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Ultrastructure of anterior lens capsule in uveitic cataract



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Purpose To evaluate the ultrastructure changes of anterior lens capsule in patients with different forms of uveitis, who underwent cataract surgery at the University Eye Clinic Ljubljana, Slovenia between years 2001 and 2007.

Methods Anterior lens capsules were obtained during cataract surgery, fixated and prepared for semi-thin sections technique and transmission electron microscopy.

Results 22 uveitic cataract were studied (idiopathic anterior uveitis-9, Fuchs-5, panuveitis-5, HSV-1, toxoplasmosis-1, uveitis in RA-1). The cataract was mature-1, cortical-14, fibrotic-1 or nuclear-6. The anterior capsule thickness showed irregularity in and between the cases spanning from 8,8 to 25,2 μ m (average 16,33, SD 4,85 μ m). In 12 samples intrusions of capsule at the capsule-epithelial border embedding cellular material were found. Capsule itself showed ultrastructure changes in 18 samples, including: filaments, rarefaction of capsule or its anterior chamber boarder, and lamellae. In 5 samples the amorphous material at the anterior chamber boarder was seen. Epithelial changes were observed in all 22 samples, including: vacuolisation, multilayered epithelium, detached epithelium, intercellular space, star-shaped nuclei, necrotic cells, lipofuscin granules. In 4 samples the epithelium was unrecognizable due to extensive fibrosis or necrosis.

Conclusion All studied uveitic cataracts showed epithelial and most also capsular changes. Some capsules showed extensive changes with unrecognizable structures. The findings did not correlate to clinical appearance or diagnosis and most probably resulted from nonspecific response to inflammation. Amorphous depositions could be inflammatory debris. Further studies need to be done to explain the underlying processes.

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Wiskott-Aldrich syndrome: A case with scleritis

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Purpose To present a case of scleritis in a patient with Wiskott-Aldrich primary immunodeficiency.

Methods An 18 year old male presented with red left eye. The patient received immunoglobulins for his immunodeficiency regularly. Dexamethasone eye drops were prescribed. In two days the redness increased and the eye became painful mainly nasally with a slight diminution of visual acuity(VA).

Results Aqueous flare and cells were detected. Intraocular pressure (IOP) was 12 mmHg. Steroid eye drops (dexamethasone) in frequent instillation and diclofenac eye drops were administered. Acyclovir (per os 800mgrx5) was added. Seven days later the redness increased and gradually the eye became more painful (nasally). Prednisolone 2, 5 % (1x1 at night) was added and also naproxen tablets (250mgrx3). Within 2 days the aqueous flare and cells ameliorated. Four days later a marginal corneal infiltration appeared nasally. Within the next week an increase of the lateral scleral involvement appeared (the nasal part was obviously better) and this lateral scleral area became painful. The fundus examination revealed chorioretinal involvement corresponding to the lateral scleral painful area. The visual acuity and the IOP remained normal. The patient was instructed to continue his therapy. He also received gamma globulins regularly (100mg/Kg/week). Only after the lateral deterioration of scleritis an additional dose was added in the middle of the week. The laboratory findings were indicative of inflammation.

Conclusion The topical treatment with dexamethasone, diclofenac and prednisolone in conjunction with acyclovir and naproxen per os was proved adequate treatment for this particular patient who also received gammaglobulins.

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Intracameral amphotericin B in the management of Candida glabrata anterior uveitis after penetrating keratoplasty

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Purpose To report management of Candida glabrata anterior uveitis after penetrating keratoplasty with intracameral amphotericin B.

Methods Case report and literature review

Results We report a case of a 79-year-old woman that presented with anterior uveitis and a dense endothelial plaque at the graft-host junction in her only eye six weeks after repeated penetrating keratoplasty with donor tissue that was culture-positive for Candida glabrata. Anterior chamber tap was performed and 5 µg of amphotericin B was injected in the anterior chamber. Cultures of the anterior chamber were positive for the same pathogen as the corneal donor rim. She was also treated with topical 0.5% amphotericin B for the next month and topical corticosteroids. At the end of follow up after 16 months her SCVA was 0.6. There was no evidence of recurrent fungal intraocular infection and the graft remained clear with the endothelial cell density of 2286 cells/mm.

Conclusion In our opinion intracameral amphotericin B is effective and safe in the therapy of Candida glabrata anterior uveitis after penetrating keratoplasty.

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The role of VEGF in intermediate uveitis patients



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Purpose Intermediate uveitis (IU) represents a chronic type of uveitis which usually manifests at young adult age with the vitreous and peripheral retina as the major sites of the inflammation. The cause and pathogenesis of IU are not known. Cystoid macular edema (CME) is the main cause of permanent visual loss in IU. Cytokines and chemokines play a major role in the pathogenesis and persistence of intraocular inflammation. The era of anti VEGF biologics opens the new possible way of treatment of uveitic macular edema of which scarce reports are found until now.

Methods Prospective cohort study. We compare the intraocular VEGF profiles in 30 patients with IU with various stages of inflammatory activity and 10 healthy controls with cataract and no uveitis in whom the AqH samples were collected during cataract surgery. Comparison was also made between the 7 IU patients with and 23 IU patients without cystoid macular edema; for 10 IU patients with active and 20 with nonactive IU. Concentrations were measured by multiplex immunoassay method in the aqueous humor.

Results There were no statistically significant differences IU patients/control group (P= 0.293); for the IU with/without CME (P=0.666);for active/not active IU (P=0.502).

Conclusion Although there are many known and unknown factors influencing vascular permeability in macular edema there is a question of reasonableness of treating the CME with intravitreal anti VEGF in IU patients with CME.

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Diffusion tensor magnetic resonance imaging and fiber tracking of trigeminal nerves in relapsing herpetic keratouveitis

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Purpose To study the trigeminal fibres (TGFs) using diffusion tensor magnetic resonance imaging (DTMRI) in relapsing herpetic kerato-uveitis.

Methods The TGFs of 11 patients (average age: 57+/-9 years), addressed for relapsing unilateral kerato-uveitis related to Herpes simplex virus (HSV) or varicelle-zoster virus (VZV), were tested using DTMRI. The diagnosis of HSV- or VZV-related disease was based on PCR or specific antibodies production within the aqueous humor, or on medical history (zoster). At the time of DTMRI, the kerato-uveitis was in a quiescent period (no recent increase of inflammation). Data were retrospectively assessed by a blinded examiner to compare the affected-side with the non-affected one and with data from sex- and age-matched normal subjects.

Results The DTRMI processing showed a significant difference in the mean isotropic fraction within trigeminal fibres innervating the affected side, compared to the non-affected side (0.41 versus 0.49, p = 0,003). There also was a trend in difference between results of the affected side of patients and those of control subjects (0.41 versus 0.44, p =0,12), whereas it was not the case for DTRMI results of non-affected sides (p=0.23).

Conclusion DTMRI is an emerging technique that allows an accurate analysis of neuronal networks in patients. Our results suggest that multiple HSV or VZV reactivation episodes induce persistent trigeminal fibres injuries (as shown by reduction of the mean isotropic fraction), that probably reflects a significant decrease in the amount of nerve endings.

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Interest of QuantiFERON®-TB (QT) Gold test (Cellestis) in patients with positive Tuberculin Skin Test (TST) in etiologic and therapeutic work-up in uveitis

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Purpose Define the interest of QuantiFERON®-TB Gold test (Cellestis) in patients with positive Tuberculin Skin Test (TST) to improve etiologic and therapeutic work-up in uveitis.

Methods All patients followed in the Ophthalmology Department of Bicêtre Hospital between April 2007 and March 2009 for uveitis had an extensive work-up including Tuberculin Skin Test (TST). Among them, 14 patients had a very positive TST making suspect an active tuberculosis infection. Then all of these patients had systematically a QuantiFERON®-TB Gold test. Finally, charts from clinical exam and therapeutic management were retrospectively analysed.

Results The mean age at the time of presentation was 58 years (+/- 9) and sex ratio (F/M) was 43%. Uveitis was bilateral in 43% of cases, and the location of inflammation was anterior in 43% of the cases, intermediate (21.4%), posterior (21.4%) or total (7%). The average of PPD test was 21.75mm (+/- 4mm, 15-65mm) and minimal delay between PPD and QuantiFERON®-TB Gold test was 3 days (mean: 42 days +/- 17). QuantiFERON®-TB Gold test was positive in 9 patients (64.3%) enjoining introduction of a full anti-tuberculosis therapeutic treatment in 8 patients (57.1%), that preceded oral steroids regimen in 42.8% of cases. Visual acuity increased dramatically (6 lines) in 4 patients and was unmodified for the others.

Conclusion QuantiFERON®-TB Gold test seems usefull in systematic diagnostic strategy in uveitis; it stays negative in 35.7% of the case suggesting that positive results are significant. However, further studies are necessary to define its place in diagnostic procedure in uveitis.

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Outcome following ranibizumab therapy for neovascular age related macular degeneration in patients with poor, intermediate and good vision

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Purpose To assess the visual outcome following intravitreal ranibizumab in eyes with wet age related macular degeneration(AMD) in patients with three different baseline vision viz; <35, 35-54 and >54 ETDRS letters.

Methods This study assessed the ETDRS visual acuity at diferent time points in a total of 98 patients.Optical coherent tomography and fundus fluorescein angiography FFA was carried out at baseline and thereafter when required. Intravitreal ranibizumab was administered monthly for 3 months and when necessary as guided by vision and OCT. Follow up period was 6 months.

Results In the 27 patients with baseline vision < 35 letters, the mean change in vision at 6 months from baseline is +8 (p=0.04). None of the patients lost more than 15 letters (floor effect) and 35% gained more than 15 letters. In 43 patients with baseline vision 35-54 letters the mean change in vision at 6 months is +10 letters(p=0.0003). None of the patients lost more than 15 letters or gained more than 15 letters.Twenty-eight patients baseline vision > 54 letters had a mean change in vision at 6 months of +6 (p=0.4). Only 16% gained more than 15 letters(ceiling effect).All the three groups were observed to have improvement of vision in the 3rd month which was sustained in the 6 month. Patients with baseline vision more than 54 letters receive an average of 3.52 injections over the 6 month period. Those with vision 35-54 and <35 received an average of 3.42 and 3.56 injections respectively over the same period.

Conclusion Response to ranibizumab in patients with good and intermediate and poor vision at baseline was similar to the ANCHOR and MARINA studies up to 6 months.

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Ocular hypertension and uveitis: about 103 cases of uveitis

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Purpose To determine the prevalence of intraocular hypertension (IOH) in 103 patients referred for uveitis in a tertiary care centre.

Methods 103 consecutive patients referred to our department for uveitis were included. Files were retrospectively analyzed for age at time of presentation, gender, type of uveitis (as defined by International Uveitis Study Group) and etiology, time of IOH (primary or secondary to treatment) and filtering surgical procedure associated. IOP was measured using Goldmann aplanation tonometry and IOH was defined as intraocular pressure ≥ 21mmHg.

Results Among all patients, 36% of uveitis were related to a concomitant infectious disease (herpetic disease in 20% of cases and herpes-zoster in 4%). No significant difference on age, gender was found between all 103 cases of uveitis. At least on episode of IOH was found during the acute phase of the disease in 27 patients (26.2%): anterior uveitis represented 74% of patients. Most of hypertensive cases were associated to herpetic disease (37%), than undetermined etiology in 6 patients (22%), toxoplasmosis uveitis in 2 patients (7.4%). Topical steroids and IOH were associated in 2 patients (7.4%). The IOH was controlled by topical hypotensive treatment in 74.1% of patients, and a filtering surgical procedure was found necessary in 7 patients (25.9%).

Conclusion This retrospective study confirms that IOH is a major complication of uveitis, especially in those involving the anterior chamber of the eye and/or related to viruses. Most of cases responded rapidly to combined topical steroids / antiglaucomatous therapy.

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Wet AMD treatment with ranibizumab - structural and functional changes during treatment



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Purpose To analyse the changes of central retinal thickness and visual acuity in patients with subfoveal neovascular membrane due to wet age-related macular degeneration who have been treated with intravitreal ranibizumab 0.5mg injections.

Methods Ten patients with wet AMD received monthly treatment with ranibizumab (Lucentis). All patients have recieved three or more injections up till now.Measurement of central retinal thickness was done by using optical coherence tomography (OCT). All injections were done in P.Stradins University Clinical Hospital, Riga during October 2008 - May 2009.

Results Eight female and two male patients with active subfoveal neovascular membrane were observed. Age of patients was from 62 up to 84 years (mean age 75 years).Mean central reinal thickness before treatment was 395 ±166µm and visual acuity 0.3 ±0.17. A month after the first injection mean central retinal thickness was 283±56µm (mean improvement by 111,5µm) and visual acuity 0,34±0,2.Mean central retinal thickness after the third injection of ranibizuamab was 222,4±62µm (reduction by 172,7µm compared with baseline findings) and visual acuity was 0,37±0,28 (mean improvement by 0,05 (in one case even by 0,3)).

Conclusion Our results of intravitreal injection of ranibizumab show improvement or stabilisation of visual acuity and reduction of central retinal thickness in all cases. The major improvement in most cases we observe after the first injection and stabilisation after following injections.

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Anti-VEGF therapy and influence of vitreomacular traction in wet AMD

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Purpose The aim of this study is to describe the results of anti Vascular Endothelial Growth Factor (VEGF) therapy in Wet Age Macular Degeneration (Wet-AMD) and the possible rol of vitreomacular traction (VMT).

Methods Descriptive, retrospective, interventional case series study of Wet-AMD patients diagnosed by angiography (AFG) and optical coherence tomography (OCT) and treated with anti-VEG. All patients received an initial charge course of three doses, and consecutive new doses if required by monthly follow up. Vitreomacular status was evaluated by OCT. Inactivation was defined by the absence of new subretinal fluid on OCT and AFG. Minimum follow up of six months after the last dose.

Results Of 76 treated patients, 27 met the inclusion criteria, 12 men and 15 women with a mean age of 79,9 years (standar deviation of 6,2). 11 had VMT and 16 had not. 48% (13/27) of patients became inactive after initial charge course, 18% (5/27) after initial good response became active despite consecutive additional doses and 33% (9/27) required more dosis but finally achieved inactivation, but did not improve visual acuity. Between patients with VMT there were more prevalence of patients who needed seven or more doses to stabilize the disease (p=0,003).

Conclusion Consecutive dosis of anti-VEGF after inicial charge course are needed to inactivate one third of patients, but these patients have poorer funtional results. VMT seems to be a bad prognosis factor.

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Intravitreal bevacizumab for neovascular age-related macular degeneration: twelve months of follow up

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Purpose To evaluate the efficacy and safety of intravitreal bevacizumab for managing choroidal neovascularization (CNV) due to age-related macular degeneration (AMD) at one-year of follow-up.

Methods A retrospective review of all patients treated with intravitreal bevacizumab (1.25mg in 0.05ml) for subfoveal or juxtafoveal CNV from AMD between June 2006 and December 2008. The files were reviewed for background data, visual acuity, fluorescein angiography, central retinal thickness and complications. Patients received injections every 4 to 6 weeks until resolution of CNV activity.

Results A total of 72 eyes of 67 patients were treated. Mean follow-up was 14.1 months (range 11 to 16 months). Twenty seven percent of patients had undergone previous PDT treatment for CNV. The mean number of injections was 2.9. The mean best corrected visual acuity was 1.06 logMAR (range 2 - 0.1 logMAR) at baseline and improved to 0.9 logMAR (range 2- 0.2 logMAR) at the last visit. The mean central retinal thickness was 404µm (range 191 - 1070 microns) at baseline and decreased to 279.9µm (range 140- 620µm) at 14-months follow-up. Statistically significant differences were found in visual acuity (p=0.044) and central retinal thickness (p=0.01) before and after intravitreal injections. No significant ocular or systemic side effects were observed.

Conclusion Our results suggest that intravitreal bevacizumab is well tolerated and is associated with improvement in visual acuity and decrease in retinal thickness at 12 months of follow-up. Further controlled, prospective and long term evaluation of this treatment is warranted.

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Evaluation and inter-observer analysis of retinography existing clinical classification system to categorize moderate retinopathies

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Purpose To evaluate existing validated systems to classify retinopathies, to determine the inter-observer agreement level

Methods 55 retinographies (both gender, >60 years old) were categorized following 3 established criteria: the International Classification and Grading System for ARM and AMD (BIRD), the Wisconsin Age-related Maculopathy Grading System (WISCONSIN) and the Clinical Age-Related Maculopathy Staging System (CARMS). The categorizing was made by 2 experts in a blind, independent way. The goal of the doubled-classification-method, with changing order no randomised, was to reject the influence of the Velo effect. Inter-observer's repeatability was checked as an agreement parameter between both Experts

Results CARMS system was chosen, since allows a global pathology classification. CARMS concordance obtained was 81.89% (expected=57.39% Kappa index=0.57). Drusen concordance obtained was 87.27% (expected=60.31% Kappa index=0.68). Pigmentation concordance obtained was 76.36% (expected=54.46% Kappa index=0.48). The reliability measured by Kappa index followed the rule: 0.80-1.00 (Excellent), 0.60-0.80 (Good) y 0.40-0.60 (Moderate), 0.20-0.40 (Low), <0.20 (Bad)

Conclusion Existing Retinopathies classification systems can be improved, to solve the huge subjective level that they present. Kappa index obtained, shown medium concordance for the inter-observer analysis, except in Drusen case, dichotomy's variable, which presented a good reliability. The worse behaviour of the Pigmentation's variable respect to Drusen variable has to be noted. This has an important influence in the global CARMS reliability, so it makes this influence to fall down

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Retinal pigment epithelial tears following administration of intravitreal bevacizumab (3 case reports)

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Purpose To report three cases of retinal pigment epithelial (RPE) tears after repeated intravitreal injection of bevacizumab for the treatment of neovascular age-related macular degeneration (AMD). To present fundus photography, fluorescein angiography (FA) and optical coherence tomography (OCT) findings.

Methods Out of 433 patients treated with intravitreal bevacizumab injections for choroidal neovascularization, three patients with vascularized RPE detachment developed RPE tear. Case 1: the patient developed RPE tear shortly after the third intravitreal injection into the left eye; cases 2 and 3: the patients developed RPE tear after a single injection into the right eye and left eye, respectively. Visual acuity testing, fundus photography and OCT were performed for all patients monthly for over a year after the first injection.

Results The preoperative size of the RPE detachment was 9.14 sq. mm for case 1, 5.16 sq. mm for case 2 and 5.93 sq. mm for case 3. At diagnosis of RPE tear, the patients suffered vision loss of three lines in cases 1 and 2 and two lines in case 3. After further treatment with bevacizumab, visual acuity at 12-month follow-up improved by two lines in case 1 and one line in cases 2 and 3.

Conclusion

1. RPE tear can occur following treatment with intravitreal bevacizumab and is associated with significant visual loss.
2. Large RPE detachment may be a predictor for RPE tear.
3. Visual acuity improved in all three cases after further treatment with bevacizumab following RPE tear.

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Retinal angiomatous proliferation: is intravitreal ranibizumab therapy enough?

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Purpose To report the use of Ranibizumab monotherapy versus Ranibizumab combined with additional photodynamic therapy (PDT) in the management of retinal angiomatous proliferation (RAP).

Methods The data from a non-randomised series of 25 patients with RAP lesions attending the Bristol Eye Hospital age-related macular degeneration (AMD) treatment clinic from April to October 2008 was analysed. The RAP lesions were classified according to Yannuzzi's system as stage 1, 2 or 3 based on clinical, ocular coherence tomogram and angiographic features. Two treatment regimes were identified (1) Lucentis monotherapy and (2) Lucentis monotherapy with additional photodynamic therapy (PDT). The main outcome measures were the % of eyes losing < 15 letters, % of eyes gaining ≥ 15 letters and the mean change in visual acuity.

Results Mean LogMAR visual acuity was 52.8 letters before treatment. Mean follow-up time is 9.5 months. Fourteen eyes had been treated with PDT before or after the use of Ranibizumab. Two eyes received PDT+IVT only (Stage 2 patients). 77% of eyes had an improvement in visual acuity. 18.5 % of eyes have had improvement of 15 or more letters. Mean change in visual acuity was +7.2 letters. 57% and 60% of patients with stage 1 & 2 disease respectively, received PDT.

Conclusion Combined therapy is effective in improving visual acuity and reducing central retinal thickness (CRT) in all patients with stage 1 lesions and most of patients with stage 2 lesions. Combined therapy offered greater gain in visual acuity in all treated patients. Stage 1 and 2 patients benefit more from the treatment if the condition is recognised and treated early.

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Apoptotic activity of intravitreal bevacizumab on rabbit retina

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Purpose To evaluate the safety and the apoptotic activity of intravitreal (IV) injection of bevacizumab in rabbit eyes by histopathological analysis.

Methods Fourteen rabbits were divided into three groups, 8 rabbits in the first and 3 rabbits in each of the second and the third groups. We applied 1.25 mg/0.05 ml IV bevacizumab to the right eyes of each subject in the first and the second groups and the same volume of saline to the left eyes of each subject in the first and the third groups. The left eyes in the second group and the right eyes in the third group were left untreated and used as the control group. After 14 days, all animals were sacrificed and all eyes were enucleated for histological examination.

Results There was no evidence of ocular inflammation, cataract, or retinal toxicity in any of the sections examined by light microscopy. We observed inflammatory cell infiltration that includes plasma cells in 3 of 11 eyes which received bevacizumab, 4 of 11 eyes which received saline, and 2 of 6 eyes which were left untreated. In all groups, we observed some vacuolization in the ganglion cell layer and some distinctive dispersion and detachment of retinal layers, including rod and cone cells and the retinal pigment epithelium. After immuno-histochemical staining with Caspase 3 and p53, there was no histological evidence of toxicity to the retina and the optic nerve in any of the sections that were analyzed in all three groups.

Conclusion According to the results of this study, IV injection of bevacizumab with the dose of 1.25 mg/0.05 ml caused no histological sign of toxicity or apoptotic activity on rabbit retina.

Commercial interest

■ 426 / 3317

Combined 23-gauge sutureless pars plana vitrectomy (ppV), injection of recombinant tissue plasminogen activator (rtPA), expansile gas and bevacizumab treating acute subretinal haemorrhage (SRH) in exudative AMD

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Purpose To evaluate the effect of 23-gauge core ppV with consecutive intravitreal injection of rtPA, gas and bevacizumab for the displacement of SRH in patients with exudative age-related macular degeneration (AMD).

Methods The retrospective, non-randomized case study included 15 eyes of 15 patients aged 74 to 89 with SRH resulting from exudative AMD. Each patient was treated with 23-gauge ppV and injection of 0.05 ml rtPA (50 µg), 0.3 ml of sulphur hexafluoride (SF6) gas and 0.05 ml bevacizumab (1.25 mg). Visual acuity (VA), lesion size measured by fluorescein angiography (FA) and retinal thickness as determined by optical coherence tomography (OCT) were evaluated pretreatment as well as 1, 3 and 6 months postoperatively.

Results The mean visual acuity pretreatment was 20/400. The figure remained constant until month 3 postoperatively. At 6 months, the mean VA had improved to 20/200. No patient had reading ability before treatment. 1 month postoperatively reading VA was achieved in 6%, rising to 27% at 6 months. There was a reduction in retinal thickness in 8 patients measured by OCT. FA showed a reduction of lesion size in 10 out of 15 patients.

Conclusion The combined application of rtPA, gas and bevacizumab via 23-gauge PPV for the treatment of SRH secondary to exudative AMD appears to be a safe and effective method. In our study, the combined treatment including the injection of bevacizumab enabled 27 % of patients to achieve reading ability at 6 months postoperatively.

■ 428 / 2417

Intravitreal bevacizumab as primary local treatment for choroidal neovascularization secondary to uveitis

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Purpose To report short term results of intravitreal (IVT) bevacizumab as primary local treatment for choroidal neovascularization (CNV) secondary to uveitis.

Methods Files of uveitic patients receiving one or more 1.25mg/0.05ml IVT bevacizumab treatment for CNV were reviewed for clinical findings, best-corrected visual acuity (BCVA), fluorescein angiography (FA) and optical coherence tomography (OCT), concurrent treatments, number and frequency of IVT bevacizumab and treatment related adverse events. Patients previously treated with any local therapy were excluded.

Results 15 patients included. Underlying diagnosis: multifocal choroiditis and panuveitis in 7, amipiginous choroiditis in 2, and for 6 remaining, serpigino choroiditis, sympathetic ophthalmia, Vogt-Koyanagi-Harada syndrome, punctate inner choroidopathy, tuberculous uveitis and idiopathic inflammation respectively. Subfoveal neovascularization in 13 eyes, peripapillary in 2. No active intraocular inflammation by time injections were given. 86.66% had a significant decrease in central foveal thickness by the end of follow-up (mean pre-treatment OCT 239,06µm, mean post-treatment OCT 195,2µm). BCVA improved in 80% of eyes at last follow-up (mean pre-treatment BCVA logMar 0,53; mean post-treatment BCVA logMar 0,29). 12 eyes received more than one IVT bevacizumab, mean number of injections in this group was 4,25(2-8), with a frequency of 1 injection every 13,68 weeks. No adverse effect related to bevacizumab nor to the injection procedure. Median follow-up 15,93(2-25 months).

Conclusion IVT bevacizumab was safe and effective first local treatment for inflammatory CNV, in patients under adequate control of intraocular inflammation.

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Photodynamic therapy for symptomatic circumscribed choroidal hemangioma

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Purpose Photodynamic therapy (PDT) is a well established treatment option for symptomatic choroidal hemangioma. We present seven patients treated with PDT at our department.

Methods Seven patients (2 female, 5 male) with a mean age of 46 Years (range 44 - 69 years) have been treated between October 2005 - March 2007. Best corrected visual acuity at first visit was minimum 0.25 and maximum 0.8 (Snellen). Indirect ophthalmoscopy showed a choroidal hemangioma with subretinal fluid. Ancillary examinations included angiography, OCT and A- and B-scan ultrasonography. Largest basal diameter was 8 mm (median; range 5.5 - 10.2 mm) and tumor height 3 mm (median; range 2.2 - 4 mm). One or more PDTs had to be done. Median follow up was 19 months (range 3 to 39 months).

Results In 3 patients one PDT was sufficient to obtain good and stable visual acuity. Three patients needed a second PDT twelve months after the initial treatment. In one patient six PDTs were necessary over a period of 20 months and additional vitrectomy had to be done. Five patients showed a gain in visual acuity (median 4 lines; range 1 to 6 lines). One patient showed unchanged visual acuity and one patient showed a loss of 3 lines after six PDTs and vitrectomy.

Conclusion In six out of seven patients stable visual acuity could be obtained. In one patient multiple PDTs and vitrectomy were necessary to achieve stable clinical results. Our findings confirm that PDT is an effective treatment modality for choroidal hemangioma.

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Repeatability in the measurement of macular pigment optical density using the Metropsis Test.

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Purpose This study was designed to report the reproducibility of macular pigment optical density (MPOD) assessed with the Metropsis Test.

Methods In two separate sessions, we measured macular pigment (MP) optical density at the fovea in 22 healthy young subjects (21.4 ± 2.26 mean age) with the Metropsis Test (Cambridge Research Systems) that is based in the apparent motion photometry method and employs a CRT monitor for stimulus presentation. The reliability was estimated by the Bland-Altman statistical method and Student's t-test for paired data. The factors determined were mean difference and the coefficient of repeatability ($COR = 1.96 \times SD$).

Results The mean MPOD in right eyes at the first visit was 0.347 ± 0.20 and 0.348 ± 0.25 at the second. The mean difference between sessions was -0.0009 ± 0.183 without statistical significance ($p < 0.382$); and with a normal distribution. The coefficient of repeatability was ± 0.358 .

Conclusion The mean difference of MPOD values was practically zero, but if we keep in mind the ends of the interval we must conclude that Metropsis Test presents a moderate repeatability in the measurement of macular pigment optical density.

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Comparison of corneal thickness, anterior segment depth and pupil diameter between patients with neovascular age-related macular degeneration and controls

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Purpose The goal of this study was firstly to compare corneal thickness at four different locations, anterior depth and pupil diameter between patients with neovascular age-related macular degeneration and healthy individuals and secondly, to find out any possible anterior segment related risk factor for AMD development.

Methods In this prospective study 69 patients (119 eyes) with neovascular age-related macular degeneration with no history of any ocular disease or surgery were included. The 31 controls (56 eyes) were also enrolled. Informed consent was obtained from all recruited individuals. Each subject underwent complete ophthalmic examination. Galilei Dual Scheimpflug Analyzer (version 3.0, Ziemer Ophthalmic Systems AG, Switzerland), a non-invasive diagnostic system designed for analysis of anterior chamber of the eye, was used for anterior segment evaluation.

Results Comparison of corneal thickness at central, paracentral, peripheral zone and at the thinnest point revealed no statistically important difference between patients with age-related macular degeneration and controls ($P < 0.5$). There was a statistically significant difference in the mean anterior chamber depth between both groups ($P = 0.002$). We did not observe statistically important difference in pupil diameter between both group ($P > 0.5$).

Conclusion The results of our study confirmed that the corneal thickness and pupil diameter are not associated with age-related macular degeneration. Further investigations are necessary to confirm our results and to find out a possible role of anterior chamber depth as an additional risk factor for AMD development.

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Comparison of macular pigment optical density (MPOD) BETWEEN A northern European and a Mediterranean population

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Purpose To investigate the Macular Pigment Optical Density (MPOD) differences between a northern European and a Mediterranean population.

Methods MPOD was measured psychophysically using Heterochromatic Flicker Photometry in 71 healthy subjects from northern Europe (18 Dutch and 53 British). 74 healthy age-matched Cretans (Greece) underwent the same measurements according to the same protocol. None of the subjects was taking any carotenoids supplements. The comparison of MPOD between the two populations was statistically assessed with a Generalized Linear Model of regression. The effect of age and gender was taken into consideration.

Results The mean MPOD of the northern Europe population was 0.43 (CI 95% 0.39-0.47) and the mean age 45 years old (± 19). No effect of age ($p = 0.402$) or gender ($p = 0.074$) was revealed into this group. Respectively, for the Mediterranean population the mean MPOD was 0.42 (CI 95% 0.38-0.45) and the mean age 39 years old (± 17); mean MPOD of this group didn't seem to change with age ($p = 0.402$) or be affected by gender ($p = 0.511$) either. No statistically significant difference in the MPOD between the two populations was revealed from the analysis ($p = 0.455$). No sex or age effect was observed in the total population ($p = 0.893$, $p = 0.094$ respectively).

Conclusion The MPOD was compared between two populations with different levels of light exposure and theoretically different diet. Although these factors are believed to affect the levels of MPOD, the latter didn't seem to differ significantly between the two groups in the present study. Additionally, the MPOD levels weren't found to change with age in either group or in the total population. Similarly, no sex differences were observed.

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Verteporfin encapsulated in cationic liposomes as an alternative drug for PDT

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Purpose Cationic liposomes bind specifically to activated endothelial cells like tissue affected by inflammation. They can be loaded with various drugs and are used as a drug carrier system to reach a locally higher concentration of the drug. We used photodynamic therapy (PDT) to compare the established verteporfin (Visudyne) with cationic liposome-bound verteporfin (Lipovert) in laser-induced choroidal neovascularization (L-CNV) of mice.

Methods Cationic liposomes were loaded with verteporfin (resulting in Lipovert). C57BL/6 mice underwent Argon laser coagulation on day 0 (d0). Lipovert or verteporfin were injected into the tail vein on d10. The CNVs were irradiated with non-thermic red light (689 nm) 1 min (verteporfin) or 60 min (Lipovert) after injection as pre-experiments showed the accumulation peak of the drugs during that time. Animals were perfused with FITC-dextran at d12, d14 or d17. Choroido-scleral flat mounts were prepared for quantification of the CNV by measuring the area of FITC-dextran positive vessels.

Results The mean CNV areas of mice treated with Lipovert or verteporfin were clearly reduced to about half of the control. Lipovert was slightly more effective than verteporfin.

Conclusion In our experiments, Lipovert can be substituted for verteporfin. Therefore, it has the potential as a drug for photodynamic therapy. As Lipovert accumulates specifically at the sites of activated endothelial cells, it may show less side effects.

■ 435

Intravitreal bevacizumab for choroidal neovascularization secondary to angiod streaks: one year follow-up

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Purpose To evaluate the efficacy and safety of intravitreal bevacizumab at one year follow-up, for the treatment of choroidal neovascularization (CNV) associated with angiod streaks.

Methods Consecutive series of 16 eyes with subfoveal or juxtafoveal CNV secondary to angiod streaks, treated with intravitreal bevacizumab (1.25 mg in 0.05 ml) between January 2007 and March 2008, were reviewed retrospectively. Ophthalmic examination including best corrected visual acuity (BCVA), optical coherence tomography (OCT) and fluorescein angiography, was performed before and after treatment. Retreatment was given every six weeks in case of persisting symptoms or CNV activity on OCT. The main outcome measures were changes in BCVA and central retinal thickness on OCT at the 1-year follow-up.

Results The mean number of injections was 4.6 at one year. Ten eyes (62.5%) received more than five injections. The mean BCVA at baseline was 20/81 (range 20/400 to 20/32) and improved to 20/40 (range 20/100 to 20/20) at one year (p=0.055). The BCVA improved by three or more lines in ten eyes (62.5%) and remained within two lines of baseline in six eyes (37.5%). Mean central retinal thickness was 404.2µm at baseline (range 160 to 602 µm) and decreased to 293.3µm (range 150 to 490µm) at one year (p=0.006). No ocular or systemic complications were noted.

Conclusion The one-year outcomes suggest intravitreal bevacizumab to be a promising treatment for CNV associated with angiod streaks, resulting in both functional and anatomical improvements. Repeated injections are needed to maintain these results. Further controlled, prospective and long term evaluations of this treatment are warranted.

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Effects of photodynamic therapy on retinal vessels in the mouse model of laser-induced choroidal neovascularization

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Purpose Primary treatment of mouse eyes with argon laser and subsequently photodynamic therapy (PDT) with verteporfin 10 days after results in a destruction of retinal structures. Our study analyses the preconditions of this effect and examines its potential benefit to a model for retinal angiogenesis.

Methods Eyes of adult mice were treated with argon laser in three areas of the eye background to induce CNV formation. Two of these areas additionally got PDT 10 days after. The mice obtained a perfusion with fluorescein isothiocyanate (FITC) dextran after another 4 days. Retinal flat-mounts were stained with lectin.

Results Areas without laser-treatment before PDT showed no lesions in retinal vessels, whereas in areas that underwent a combination of laser-treatment and PDT destructions of retinal capillary vessels cropped up. Conditions for this effect were optimized.

Conclusion Laser-treatment seems to be prerequisite for a destructive effect on retinal capillary vessels by means of a following PDT.

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Macular edema and neovascularization in a case of Stargardt macular dystrophy

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Purpose To present a case of macular edema and neovascularization in a patient who was diagnosed with Stargardt macular dystrophy.

Methods We present the case of a 22 year old male who experienced visual deterioration in his left eye. At presentation his visual acuity was OD 8-9/10 and OS 6-7/10. OCT scan, fluoroangiography (FA) and electroretinography (ERG), were performed.

Results OCT scan revealed cystoid macular edema in the right eye, and increased reflectivity of the pigment epithelium and some subretinal fluid in the left eye. FA findings were consistent with Stargardt dystrophy. However in the right eye an area of increasing hyperfluorescence was observed (presence of neovascularisation). ERG: subnormal photopic and scotopic responses. The color vision was impaired mainly in the left eye. Following the experience of other investigators acetazolamide was administered orally (300 mg per day) and resolution of the cystoid edema was obvious in OCT scan after 20 days. The visual acuity increased (OD 10/10 and OS 8/10). Two weeks after the discontinuation of acetazolamide the macular edema reappeared and the visual acuity diminished: OD 8-9/10 and OS 6-7/10.

Conclusion This is a peculiar case of cystoid macular edema and neovascularization in Stargardt macular dystrophy. Our findings suggest, in consistency with other investigators, that administration of oral acetazolamide may be beneficial for the resolution of macular edema. The neovascularization in the left eye remained stable during the follow up period and it was considered that any intervention was not necessary at that time.

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Some questions of immune response to retinal S-antigen and development of myopia's complications

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Purpose To estimate reliability of the prognosis of an unfavorable current of myopia on the basis of estimation immune response to a retinal S-antigen in tears and blood sera

Methods We have studied an autoimmune response to S-Antigen (48kDa) in tears and in blood sera (ELISA) in 113 patients with progressive and complicated myopia with a follow-up study of 44 patients (average age 11 ± 1.5 y.o.) without (15) and with (29) different forms of peripheral vitreochorioretinal dystrophies (PVCRD) during 11 years.

Results The long-term study shows, that prognosis of complication's development was right in 83,4%. Most unfavorable signs of preclinic myopia complications are a deficient of S-antibodies (s-ab) in tears and blood sera - the prognosis for development of retinal disorders and/or fast and long myopia progression was confirmed at 51,6% of patients. High level of s-ab in blood sera and deficient of s-ab in tears was also the sign of development of PVCRD (the prognosis was confirmed at 22,6%). Occurrence or aggravation of changes at the retina began between 6 month and 1,5 years after revealing of the specified changes that testifies to authentically early prognosis in the beginnings of development of preclinical changes in myopic eye. Patients with tuberculin sensibilisation and deficient of S-antibodies in blood sera (observation's time 14 years) had worse retinal disturbances.

Conclusion This data allows to use s-ab level for prognosis of the development of myopia's complication. It is very important to know that tuberculin vaccinations may be a risk-factor in development of myopia complications and autoimmune uveitis. It opens up new vistas for the immunocorrective methods in the system of the treatment of progressive and complicated myopia.

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Bevacizumab and ranibizumab treatments for neovascularization from causes other than age-related macular degeneration

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Purpose To report the results of intravitreal bevacizumab (Avastin) and ranibizumab (Lucentis) treatment for ocular neovascularization from causes other than age-related macular degeneration.

Methods We performed a retrospective case note analysis of eyes who received intravitreal bevacizumab and ranibizumab for patients with ocular neovascularization. Repeated treatment occurred if there were signs of recurrence of disease activity. The main outcome measures were visual acuity, clinical signs and findings on optical coherence tomography and fluorescein angiography.

Results Nine patients (10 eyes) were included in this study. Diagnoses included subfoveal choroidal neovascularization secondary to punctate inner choroidopathy (n=2), myopic degeneration (n=2), pseudoxanthoma elasticum (n=1). Others have proliferative diabetic retinopathy (n=2), central retinal vein occlusion (n=1), clinically significant macular oedema (n=1), Coat's disease (n=1). The range of baseline visual acuity was from counting fingers to 6/9. The mean follow-up was 12 months; the mean number of injections in 9 eyes was 1.5. One eye had 12 injections. At the last follow-up, 9 eyes had improved or stable visual acuity. There were no complications reported.

Conclusion Ocular neovascularization secondary to non AMD causes treated with intravitreal bevacizumab and ranibizumab responded favourably, despite varying underlying aetiologies.

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Treatment of neovascularization myopic with ranibizumab: 18-month results

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Purpose To evaluate the efficacy of intravitreal Ranibizumab as treatment for choroidal neovascularisation due to pathological myopia

Methods A retrospective, non-comparative study of 21 eyes treated with intravitreal injections of Ranibizumab. Ten eyes had been treated previously with photodynamic therapy and eleven received Ranibizumab as first therapy. After thorough ophthalmologic examination, fluorescein angiography (FAG) and optical coherence tomography (OCT) intraocular injection was practised, a new treatment was decided according to the subjective impression of the patient, visual acuity, presence or absence of metamorphopsia, biomicroscopy and OCT examination.

Results Twenty one eyes from 17 patients were finally included. Patients were followed up for 18 months. The mean age at initial treatment was 55.71 years. Mean refractive error was -12.6 diopters. Regarding AGF all neovascular membranes were classical and sub or juxtafoveal localised. At the end of the 18 months after treatment 16 eyes (76.19%) showed better visual acuity ranging from one or more lines on Snellen chart, 10 eyes (47.61%) improved two lines or more, 4 eyes (19%) did not show any changes and one eye (4.76%) worsened two lines. At 18 months the mean best-corrected visual acuity (BCVA) improved from 0.28 to 0.50 (p= 0.001). The mean central macular thickness decreased

Conclusion Ranibizumab may be a good therapeutic option as treatment for choroidal neovascularisation due to pathological myopia, it improved visual acuity and anatomical features, even in non-responders to photodynamic therapy.

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Comparison of spectral-domain cirrus optical coherence tomography (OCT) and time-domain Stratus OCT for the evaluation of macular and peripapillary retinal nerve fiber layer thickness in patients with diabetes type 2

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Purpose To compare Cirrus Fourier-domain OCT with Stratus Time-domain OCT for measuring the peripapillary retinal nerve fiber layer (RNFL) and macular thickness in patients with diabetes mellitus type 2 without diabetic retinopathy.

Methods Prospective study of 49 patients with diabetes type 2. Only one eye by each patient was randomly included in the study. All of them were underwent three optic disc cube 200x200 scans and three macular 512x128 volume cube scans with the Cirrus HD-OCT system (Carl Zeiss Meditec, Inc.) and with the Stratus system. The average of all the scans was calculated. The RNFL thickness average, the RNFL thickness at each quadrant, and each 12 clock-hours position were evaluated comparing the two devices. The macular values of the nine areas corresponding to ETDRS protocol were compared between both systems.

Results 21 men (42.9%) and 28 women (57.1%) were included in the study. Mean age was 60 (42-72 years). The average of the macular thickness using Cirrus HD-OCT was approximately 66.37 µm higher the average measured with Stratus (263.52 µm vs 197.15 µm; p=0.001). We did not find statistically significant differences between Cirrus and Stratus in RNFL average thickness measurements. Statistically differences were only found in one clock-hour position.

Conclusion Macular measurements using Cirrus OCT are higher than those measured with Stratus OCT in patients with diabetes mellitus type 2. It doesn't exist a clear difference in RNFL measurements between two devices. Our results are consistent with other previous studies obtained in healthy subjects in similar conditions.

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Differences in macular thickness studied by time domain optical coherence tomography (OCT) and spectral domain OCT in healthy subjects

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Purpose To compare Stratus Time-Domain optical coherence (OCT) with Spectralis Spectral-domain OCT for measuring macular thickness in healthy patients.

Methods Prospective study. Fifty- five healthy subjects were studied. All them underwent 3 Time Domain Optical Coherence Tomograph (OCT) scanning (Stratus OCT, Carl Zeiss Meditec, Inc.), fast macular thickness scan strategy, and 3 Spectral Domain OCT (Spectralis OCT; Heidelberg Engineering, Heidelberg, Germany) with macular scan 512 x 128 volume cube. Mean values of macular thickness measurement of the nine areas corresponding with "Early Treatment Diabetic Retinopathy Study" (ETDRS) were compared between both tomographs.

Results We examined 55 healthy subjects, 43 women and 12 men, mean age was 35.64 ±13.73 years. Central thickness measured by Status OCT was 200.51 ± 17.98; central thickness obtained by Spectralis was 276.34 ± 20.71 microns. The t-test assesses whether the means of all ETDRS macular areas measurements of the two tomographs are statistically different from each other, finding that Spectralis central thickness measurements was 75.83 microns higher than using the Stratus OCT.

Conclusion Time Domain OCT uses the joint of the inner and outer segments of the photoreceptors as outer reference for macular thickness calculation whereas Spectral Domain OCT bases its reference in the retinal pigment epithelium, so differences between both of them are predictable. With our study we obtain a measurement of useful conversion to extrapolate the results obtained with a tomograph to the other one, being of great utility in the clinical practice.

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Comparison of the peripapillary retinal nerve fiber layer thickness in healthy subjects measured by spectral-domain Cirrus optical coherence tomography and time-domain Stratus optical coherence tomography

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Purpose to compare Cirrus Fourier-domain optical coherence tomography (OCT) with Stratus Time-domain OCT for measuring the peripapillary retinal nerve fiber layer (RNFL) thickness in healthy subjects.

Methods One hundred and thirty two healthy subjects were prospectively and consecutively selected. Only one eye was randomly included in the study. All of them underwent three optic disc cube 200x200 scans with the Cirrus HD-OCT system (Carl Zeiss Meditec, Inc.). The average of the 3 scans was calculated. With the Stratus OCT system (Carl Zeiss Meditec, Inc.) a fast RNFL thickness scan was recorded. The average of RNFL thickness, and RNFL thickness at each quadrant, and each 12 clock-hours position was evaluated comparing both devices. Reproducibility of RNFL thickness measurements using de Cirrus HD-OCT was studied, and the variation coefficient (COV) was calculated.

Results Mean age was 40.06 ± 13.63 years (ranged from 17 to 72 years). Thirty nine were men (29.5%) and 93 women (70.5%). The average of the peripapillary RNFL thickness was 96.16 µm and 98.27 µm using Cirrus HD-OCT and Stratus OCT respectively (p=0.001). Statistically differences were found in the temporal quadrant (p=0.026) as well as in several hour sectors. The Cirrus HD-OCT reproducibility was high, with a mean COV of 4.54% (ranged from 1.64% to 6.37%).

Conclusion Mean peripapillary RNFL thickness measured with Cirrus HD-OCT is slightly lower than with Stratus OCT.

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Comparison of Spectralis Fourier-domain optical coherence tomography (OCT) and time-domain Stratus OCT for the evaluation of macular thickness in patients with diabetes mellitus type 2

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Purpose To compare spectralis fourier-domain OCT with Stratus Time-Domain OCT for measuring macular thickness in patients with diabetes mellitus type 2 without retinopathy.

Methods Prospective study of 49 patients with diabetes type 2. Twenty-one men (42.9%) and twenty-eight women (57.1%) were included. One eye by each patient was randomly included in the study. All of them were underwent three macular 512x128 volume cube scans with the Spectralis OCT system and with the Stratus OCT. The average of all the scans was calculated. The macular values of the nine areas corresponding to the Early Treatment Diabetic Retinopathy Study (ETDRS) protocol were compared between the two systems.

Results We found statistically significant differences in all macular measures between the two systems (p<0.001). The average of macular thickness measured with Spectralis OCT was approximately 266.84 µm (± 12.22). The average of macular thickness using Stratus OCT was 194.56 µm (± 12.04). The difference of foveal values between both devices was approximately 72.28 µm (± 9.67). Retinal thickness values were higher with Spectralis OCT

Conclusion Macular measurements using Spectralis OCT are higher than those measured with Stratus OCT in patients with diabetes mellitus type 2. The results are comparable with the results obtained between Stratus OCT and Cirrus-HD OCT in the same conditions and with the same sample of patients and with the values obtained in healthy subjects.

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Repeatability of retinal nerve fiber layer and macular thickness measurements using Cirrus Fourier-domain optical coherence.

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Purpose To test intrasession, intersession, intervisit, and interoperator reproducibility of retinal nerve fiber layer (RNFL) measurements and retinal thickness using Cirrus Fourier-domain optical coherence tomography (OCT).

Methods Seventy-two eyes of 72 healthy subjects underwent three macular 512x128 volume cube centered on the fovea and three 360° optic disc circular scans done by the same observer. This sequence was repeated by another observer on a second visit within a 2-week period. Descriptive statistics, analysis of variance, intraclass correlation coefficients (ICC), and coefficients of variation (COV) were calculated for the 9 macular areas described by the Early Treatment Diabetic Retinopathy Study (ETDRS) in the macular protocol and for quadrants and RNFL clock hour sectors.

Results All measurements were highly reproducible. The ICCs ranged from 0.823 to 0.992 using macular volume cube and from 0.886 to 0.984 with optic disc cube protocol. Mean differences between both operators were lower than 3 µm in all measurements and no significant differences were found. Total retinal thickness mean was 285.17 ±15.31 µm by observer 1 and 284.20 ±12.89 µm by observer 2. Mean COV was 1.2 ±0.99% (ranged from 0.5 to 1.8%) for the nine ETDRS areas. Lowest COV was found for the nasal inner area. Mean RNFL average thickness was 95.99 ±7.67 and 95.72 ±7.87 µm by observer 1 and 2, respectively. Mean COV was 4.41 ±3.01% (ranged from 1.43 to 6.66%).

Conclusion Retinal and RNFL thickness measurements obtained using Cirrus Fourier-domain OCT show good reproducibility for healthy eyes. The device is an important tool for to the diagnosis and follow-up of retinal and optic nerve pathologies.

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Comparison of spectral-domain Cirrus optical coherence tomography and time-domain Stratus optical coherence tomography for the evaluation of macular thickness in healthy subjects

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Purpose to compare Cirrus Fourier-domain optical coherence tomograph (OCT) with Stratus Time-domain OCT for acquiring retinal images and measuring retinal thickness.

Methods Eighty five eyes from 85 healthy subjects, 26 men (30.6%) and 59 women (69.4%), aged 16 to 72 years were prospective and consecutively selected. All of them underwent three macular 512x128 volume cube scans with the Cirrus HD-OCT system (Carl Zeiss Meditec, Inc.). The average of these three scans was calculated. With the Stratus OCT system (Carl Zeiss Meditec, Inc.) a fast macular scan was recorded. The nine areas corresponding to the Early Treatment Diabetic Retinopathy Study (ETDRS) macular protocol were compared between both devices. The reproducibility of macular thickness measurements using de Cirrus HD-OCT was studied, and the variation coefficient (COV) of each parameter was assessed.

Results Cirrus HD-OCT macular thickness measurements were approximately 64.22 µm higher than those from Stratus OCT (266.40 µm vs 202.18 µm). The measurements in all nine ETDRS areas were statistically higher using the Cirrus HD-OCT. Nasal inner area showed the highest value. Retinal thickness measurements using the 512x128 macular cube scan were highly reproducible for the nine ETDRS areas. Mean COV was 1.45 ± 1.11% (ranged from 0.98% to 2.49%). The lowest COV was found for the temporal inner ETDRS area (COV=0.98%) and the highest for the central ETDRS area (COV=2.49%).

Conclusion Macular measurements using Cirrus HD-OCT are clearly higher than those obtained by Stratus OCT.

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Clinically useful wide-field high-resolution retinal imaging optics with a dual-conjugate adaptive instrument

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Purpose The aim of this project is to apply the technique of multi-conjugate adaptive optics (MCAO) for high-resolution imaging of the retina over a wide field-of-view (FOV).

Methods MCAO is the principal technique to overcome the limitations of conventional AO systems by increasing the corrected FOV. The current system incorporates two deformable mirrors (DMs). Optically, these two DMs correspond to separate planes along the optical axis of the eye. This allows the correction of field-dependent aberrations and subsequent wide-field high-resolution retinal imaging over a wide FOV. Normative data from healthy subjects was collected in order to determine clinical potential and limitations.

Results We have demonstrated the MCAO concept with a current FOV of 7x7 deg on both model and human eyes. The diffraction-limited resolution is around 2 µm on the retina, allowing most cone photoreceptors to be resolved. Retinal morphology of healthy subjects was investigated. The estimated cone separation at a retinal eccentricity of 1.2 deg and 2.5 deg from the fovea was 6 µm and 9 µm, respectively, in agreement with the literature. The relatively narrow depth of focus enables us to image different layers of the retina. Focusing on deeper layers allows us to image the cone photoreceptor layer. By focusing on the upper layers we can also image the retinal capillary layer and nerve fibre layer with the same high resolution.

Conclusion Our instrument allows retinal features down to 2 µm to be resolved over a 7x7 deg FOV. We believe that this new technique has a future potential for clinical imaging with an impact particularly important for early diagnosis of retinal diseases.

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Retinal nerve fiber layer and macular thickness measured with Cirrus and Spectralis Fourier-domain optical coherence tomographies: comparative study in healthy eyes.

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Purpose To compare the retinal nerve fiber layer (RNFL) measurements and macular thickness measurements in healthy eyes using two different Fourier-domain optical coherence tomographies (OCT) devices: Cirrus HD OCT (Carl Zeiss Meditec, Dublin, Ca) and Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany).

Methods Fifty healthy eyes (33 women and 17 men, aged from 23 to 59 years) were prospectively analysed using 3 optic disc protocols and 3 volume cube scans with each device. Descriptive statistics, analysis of variance and coefficients of variation (COV) were calculated for RNFL average thickness and for the 9 areas corresponding to the Early Treatment Diabetic Retinopathy Study (ETDRS) in macular protocol. Differences between the acquisitions of RNFL quadrants with both devices made them unable to compare.

Results RNFL thickness measurements showed significant differences between Cirrus HD OCT and Spectralis OCT (mean: 98.91 ± 7.39 microns and 102.20 ± 7.93 microns respectively) (p<0.001). Total retinal thickness mean showed significant differences between Cirrus OCT and Spectralis OCT (mean: 269.27 ± 23.25 and 277.79 ± 20.43 microns, respectively) (p=0.032). All measurements were highly reproducible. The retinal COV were from 0.1% (nasal outer ETDRS area) to 2.3% (average thickness) using Cirrus OCT and from 0.1% (nasal outer ETDRS area) to 2.2% (inferior outer ETDRS area) with Spectralis OCT. RNFL average thickness showed lower COV using Cirrus OCT (1.36%) than Spectralis OCT (1.79%).

Conclusion Fourier domain OCTs show differences between their measurements and good reproducibility to analyze RNFL and macular thickness.

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Optical coherence tomography and biomicroscopical analysis in macular holes

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Purpose To assess the usefulness of optical coherence tomography (OCT) for better differential diagnosis of macular holes in comparison with biomicroscopical fundus analysis.

Methods We reviewed the files of 25 eyes of 24 patients who were diagnosed as having a macular hole on OCT examination and biomicroscopical fundus analysis. Each eye underwent six radial 3-mm OCT scans centered on the macula, one 6-mm vertical and one 6-mm horizontal scan. Retinal thickness was measured at the foveal center and 750 µm from the center, vertically, and horizontally. The diameter of the macular contour was also measured on vertical and horizontal scans.

Results On biomicroscopy only 8 eyes (32%) were diagnosed as having macular hole, while in the remaining 17 eyes (68%) diagnosis of macular hole was possible only performing an OCT examination.

Conclusion Optical coherence tomography is very useful in the diagnosis of macular holes compared with classic biomicroscopical analysis.

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Local and genetical determinants of vascular endothelial growth factor in vision threatening proliferative diabetic retinopathy

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Purpose To search for the association between the -634 C/G polymorphism of the VEGF gene and vision threatening proliferative diabetic retinopathy (PDR) and to determine whether vitreous levels of VEGF were affected by genetic factors.

Methods In this cross sectional case-control study 349 unrelated Slovene subjects (Caucasians) with type 2 diabetes mellitus were enrolled: 206 patients with vision threatening PDR in whom vitrectomy was performed, and the control group of 143 subjects with type 2 diabetes of duration of more than 10 years who had no clinical signs of diabetic retinopathy. In 68 out of 206 patients with PDR (71 eyes) vitreous fluid samples (0.3 ml) were obtained by vitreoretinal surgery.

Results The -634 C/G VEGF gene polymorphism was not associated with PDR. Moreover, significantly higher vitreous levels of VEGF were demonstrated in diabetics with the CC genotype compared to those with the other (CG + GG) genotypes.

Conclusion Despite the effect of the -634 C/G VEGF gene polymorphism on vitreous levels of VEGF in PDR, it failed to contribute to the genetic susceptibility to PDR.

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Visual function vs quality of life assessment in patients with laser-treated diabetic macular oedema

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Purpose To compare different visual function assessments in patients with laser-treated diabetic macular oedema (DMO) to the self-assessment of visual dysfunction and quality of life (QoL) time trade-off (TTO) utility measures.

Methods Forty patients with DMO previously treated with laser were assessed for best corrected visual acuity (distance and near), reading speed, fixation location and stability, microperimetry with Nidek MP-1, and optical coherent topography (OCT). Subjective analogue self-assessment of distance and near visual dysfunction and QoL TTO utility measures were also assessed.

Results Distance visual acuity correlate well with fixation location and stability, and central retinal thickness of OCT. Near visual acuity and reading speed correlate well with relative scotoma size. However, visual dysfunction and QoL TTO utility correlations are more complex, reading speed in the better seeing eye is best to correlate with QoL TTO utility after correcting for age.

Conclusion Laser treated DMO patients often report subjective visual dysfunction that cannot be explained by best corrected distance visual acuity. Scotoma size affects near vision and reading speed, leading to poor quality of life.

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Intravitreal combination of triamcinolone acetonide – bevacizumab (Kenacort–Avastin) in diabetic macular edema

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Purpose To evaluate the effect of intravitreal injection of Triamcinolone Acetonide – Bevacizumab (Kenacort®)-(Avastin®) in the treatment of diabetic macular edema

Methods Twenty seven eyes of 17 patients with diabetic macular edema participated in this study. Each patient received an intravitreal injection of 0,1 ml triamcinolone acetonide (Kenacort®) (0,2mg)combined with bevacizumab (Avastin®) (1,25mg). Mean age was 64years and mean duration of diabetes 15,5years. The primary outcome measures were changes in best corrected visual acuity and in central macular thickness measured by OCT. The follow up period was six months from the first intravitreal therapy.

Results During the follow up period 3 eyes(11%) received one, 18 eyes(67%) received two and 6 eyes(22%) received three intravitreal injections according to the monthly follow up examination. There were not observed any important complications of the intravitreal combination of Triamcinolone Acetonide – Bevacizumab either during or after the treatment. The mean visual acuity increased 0,12 (decimal) (p<0,0001) and the central macular thickness decreased 235µm (p=0,018).

Conclusion Intravitreal combination of Triamcinolone Acetonide – Bevacizumab seems to be effective in improving visual acuity and macular edema in patients with diabetes.

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Use of the contrast sensibility in the diagnosis of diabetic retinopathy and diabetic macular edema

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Purpose To determine the validity of the contrast sensibility, in the diagnosis of the diabetic retinopathy and macular diabetic edema.

Methods Cross -sectional observational study consisting of 628 eyes of patients with diabetic retinopathy, being realized the curve of contrast sensibility using the test CSV-1000E. There were studied 4 spatial frequencies (3,6,12,16 cycles / second). The results were analyzed for each of the frequencies, corrected by the age, degree of diabetic retinopathy and thickness macular.

Results In patients with retinopathy diabetic sensibilities of 4 were obtained (3 ciclos/seg) 2 (6 ciclos/seg) and 1,5 (12 and 18 ciclos/seg) .La average decrease of the curve corrected by the age was 30 %, 37 %, 42 % and 45 % for each of the analyzed frequencies. The patients with thicknesses macular top to 400 microns experimented decreases superior to 50 % of the curve of sensibility.

Conclusion We find a widespread reduction of the curve of sensibility to the contrast, in patients with diabetic retinopathy and diabetic macular edema. The high frequencies were more prematurely affected, being able to serve in proof of screening, since the functional damage in these frequencies might precede the structural anomaly.

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Use of retinal photography in the diagnosis of clinically significant macular edema (CSME)

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Purpose To determine the validity of the simple and stereoscopic retinal photography in the diagnosis of clinically significant macular edema (CSME).

Methods Cross-sectional observational study consisting of 420 eyes of patients with diabetic retinopathy, which was a combination of retinography (simple, light and stereoscopic aneritra), after expansion, to determine its validity in the diagnosis of diabetic macular edema. We calculated sensitivity, specificity, positive and negative predictive values and the correlation with the gold standard test (optical coherence tomography). The retinographs were evaluated by three experts and their results analyzed by the statistical program SPSS 15.0 Windows.

Results For the analysis of the clinically significant macular edema (CSME), there were obtained sensibilities near to 80 % (color photographs 45 °, green 45 °, 30 °) and superior to 80 % (stereoscopic green 45 °, stereoscopic color 30 °, stereoscopic green to 30 °), reached the stereoscopic green photography 30 °, a sensibility of 92,5 %. The specificity was 90%. The positive predictive value was greater than 95% and the negative value greater than 90% with a coefficient of agreement (80%) and a degree of consistency with the benchmark of over 80%. Less than 5% of the retinographs were of poor quality.

Conclusion The stereoscopic retinal photography to color of 45 ° and 30 °, aneritra of 30 ° and stereoscopic aneritra of 30 ° offer a sufficient sensibility and specificity to be used in the diagnosis of clinically significant macular edema (CSME).

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Long-term result of intravitreal steroid for macular oedema: 2 to 5 year follow-up of 92 eyes

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Purpose A topical area is how best to manage diabetic macular oedema (intravitreal steroid, laser, anti-VEGF). However little or no long-term data exists for steroids. We hence studied the 2 to 5-year results of intravitreal triamcinolone (IVTA) in diabetic macular oedema.

Methods A retrospective cohort of 92 eyes was recruited, inclusion criteria subjects with diabetic macular oedema injected with 4mg / ml IVTA with regular follow-up of 2 to 5 years. Exclusion criteria were subjects with non-diabetic oedema (uveitis, vascular, post-operative) and baseline foveal ischaemia at time of first injection. The following were recorded: visual acuity, central retinal thickness (OCT) prior, 1 month after (+/- 1 week) and 3 months post-IVTA (+/- 2 weeks), complications, and angiograms pre- and post-treatments. 88% had Type 2 diabetes. Mean duration of diagnosis of diabetes was 14 years, mean age 64 (29-84).

Results At 2 to 5 years IVTA continued to be effective both in improving visual acuity and reducing macular thickness in 76% of subjects, including after multiple injections. In 24% of subjects foveal ischaemia limited outcome, usually 36 to 54 months post initial treatment. In 8% of subjects a repeat injection was associated with cessation of leakage for 2 to 3 years, or a persistent marked diminution in leakage of in excess of 100 microns, thereafter vision dropping to 6/12 to 6/18 despite further injections. Intra-ocular pressure rises were controlled topically, there was one case of sterile endophthalmitis.

Conclusion IVTA continues to offer significant visual benefit and reduction in macular thickness 2 to 5 years after initiation of therapy, including after multiple injections. Therapeutic potential is limited by ischaemia in 24% of subjects.

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Ocular and systemic endothelial function in the offspring of diabetics: a pilot study

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Purpose To investigate ocular and systemic correlates of endothelial function in the normoglycaemic offspring of Type 2 Diabetics (T2DM).

Methods Healthy participants aged between 25-65 with (n=30) and without (n=39) a family history were recruited. Retinal vessel reactivity was assessed by using the Retinal Vessel Analyser (RVA, Imedos GmbH). In addition, systemic endothelial function was assessed by using the flow mediated dilation (FMD) technique.

Results Parametric testing showed no significant differences in anthropometric, blood assay or ocular and systemic function between both groups (p>0.05). The average maximum dilation in the measured retinal artery correlated significantly with the maximum dilation of the measured brachial artery (p=0.002 R=0.55) in healthy controls; however, this was not true for subjects with family history of T2DM.

Conclusion Subjects with family history of T2DM show possibly early signs of endothelial dysfunction that, in certain conditions, could contribute to the higher risk of this group of developing similar pathology to their parents.

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The relationship between retinal hemodynamics and systemic markers of endothelial function and inflammation in type 2 diabetes

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Purpose The aim of the study was to investigate the associations, if any, between retinal hemodynamics and systemic markers of endothelial function and inflammation in patients with type 2 diabetes and non-proliferative diabetic retinopathy (NPDR).

Methods Retinal blood flow measurements and blood serum were obtained from 9 healthy controls (Group 1, mean age 53.8 yrs, SD 11.7), 23 patients with mild-to-moderate NPDR (Group 2, mean age 61.4 yrs, SD 8.5; duration 9.9 yrs, SD 8.8) and 14 patients with moderate-to-severe NPDR (Group 3, mean age 60.3 yrs, SD 9.9; duration 17.0 yrs, SD 9.4). Retinal blood flow was measured in the supero-temporal arteriole using the Canon Laser Blood Flowmeter. Blood samples were collected to derive levels of intercellular adhesion molecule (ICAM-1), vascular adhesion molecule (VCAM-1), E-selectin and von Willebrand factor (vWF).

Results Diameter, velocity, maximum-to-minimum velocity ratio and flow were the same across the groups and did not change over a 6 month follow-up. A1c and ICAM-1 were significantly elevated across the groups (p=0.001 and p=0.030, resp., ANOVA). VCAM-1 (r =-0.325, p=0.046) and vWF (r =-0.334, p=0.050) showed relatively weak associations with baseline max:min velocity ratio. vWF showed moderate associations with change in velocity (r = 0.571, p=0.007) and change blood flow (r = 0.455, p=0.038).

Conclusion The relationship between endothelial dysfunction and inflammation markers and retinal hemodynamics in type 2 diabetes is complex since not all markers show an association. This work is on-going.

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Assessment of retinal arteriolar hemodynamics in patients pre- and post-cataract extraction

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Purpose We have previously demonstrated that artificial light scatter results in the erroneous elevation of retinal vessel diameter and blood flow using densitometry based techniques. The aim of this study was to determine the impact of cataract on the quantitative, non-invasive assessment of retinal arteriolar blood flow.

Methods Of the original 30 recruited patients who were scheduled for extra-capsular cataract extraction using phacoemulsification and intraocular lens implantation, ten patients between the ages of 61 and 84 (mean 73 ± 8) successfully completed the study protocol. Two visits were required to complete the study, one prior to the surgery and one at least six weeks after the surgery. Cataract status was documented using the Lens Opacity Classification System (LOCS, III) on the first visit. Retinal arteriolar hemodynamics were measured using the high intensity laser setting of the Canon Laser Blood Flowmeter (CLBF) on each visit.

Results Group mean retinal arteriolar diameter and blood flow were significantly lower following extracapsular cataract extraction (Wilcoxon signed-rank test, $p=0.022$ and $p=0.028$ respectively); however, centreline blood velocity was unchanged (Wilcoxon signed-rank test, $p=0.074$). The primary reason for failure to complete the study protocol was due to poor retinal image quality impairing CLBF measurement.

Conclusion Densitometry assessment of vessel diameter is extraneously impacted by the presence of cataract. Care needs to be exercised in the interpretation of studies of retinal vessel diameter and blood flow that utilize densitometry techniques.

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Efficacy after intravitreal bevacizumab and triamcinolone injection in BRVO patients

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Purpose To compare the efficacy of the injection after intravitreal bevacizumab and Triamcinolone(TA) in patients with macular edema secondary to BRVO

Methods A consecutive retrospective review of patients with ME secondary to BRVO whowere treated with intravitreal bevacizumab(1.25mg/0.05mL) and TA(4 mg/ml). Patients underwent complete ophthalmic evaluation, which included visual acuity testing(log MAR), OCT, and/or angiographic testing.

Results There were 53 patients who received intravitreal bevacizumab(21 eyes) and TA(32 eyes) injections. The mean length of follow-up was 13.2 months. In patients with intravitreal bevacizumab injection, the mean visual acuity(VA) improved from 0.77 ± 0.50 at baseline to 0.43 ± 0.30 ($p=0.095$) at 1 month, 0.31 ± 0.22 ($p=0.007$) at 3 months and 0.51 ± 0.26 ($p=0.299$) at last follow-up. The mean central macular thickness(CMT) was $616.0 \pm 77.0 \mu\text{m}$ at baseline and decreased to $259.0 \pm 32.0 \mu\text{m}$ ($p=0.028$), $267 \pm 62.4 \mu\text{m}$ ($p=0.029$) and $323.3 \pm 34.3 \mu\text{m}$ ($p=0.036$) at 1, 3 months, and last follow-up. In patients with intravitreal TA injection, the mean VA improved from 0.78 ± 0.36 at baseline to 0.54 ± 0.26 ($p<0.001$) at 1 month, 0.43 ± 0.28 ($p=0.450$) at 3 months and 0.63 ± 0.36 ($p=0.001$) at last follow-up. The mean CMT was $577.5 \pm 29.8 \mu\text{m}$ at baseline and decreased to $397.2 \pm 114.5 \mu\text{m}$ ($p=0.004$), $391 \pm 72.4 \mu\text{m}$ ($p=0.003$) and $412.3 \pm 123.1 \mu\text{m}$ ($p=0.034$) at 1, 3 months, and last follow-up. No adverse side effects were observed following injections.

Conclusion The observed anatomic and VA improvements and lack of serious adverse side effects after intravitreal bevacizumab and TA injection in ME. However, patients received intravitreal bevacizumab injection led to no significant difference in visual acuity and macular thickness compared to the group treated with Triamcinolone injection.

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Effects of conventional argon panretinal laser photocoagulation on retinal nerve fibre layer and driving visual fields in diabetic retinopathy

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Purpose To determine the effects of argon green panretinal laser photocoagulation (AG-PRP) on retinal nerve fibre layer thickness (RNFLT), threshold visual fields (VF), and Estermann full binocular VF over time.

Methods Prospective, pilot clinical study of patients with newly-diagnosed proliferative diabetic retinopathy (PDR). Time-Domain optical coherence tomography (TD-OCT) of optic nerve head and 24-2 SITA-Fast Humphrey/Estermann VF (HVF, EVF) recorded at baseline, 10 weeks and 6 months post-laser. Quantitative field analysis of central 10°, 24°, and binocular VF.

Results 10 eyes underwent uncomplicated multiple-session 100ms AG-PRP using 2000 burns, 400µm spot, and mean power 136 mW (SD 39.3). TD-OCT detected and quantified an increase in mean RNFLT at 10 weeks (+8 µm; $p<0.05$) and progressive thinning at 6 months (-4 µm; $p<0.05$) compared to baseline. Mean threshold sensitivities, 10° and 24°, improved at both time-points in the majority (9/10 and 8/10) of patients. Masked grading of EVFs showed no significant change with treatment.

Conclusion This pilot study demonstrates that conventional AG-PRP may increase the RNFL in the short-term, presumably related to laser-induced axonal injury, with progressive thinning of RNFL in the longer-term. The sensitivity of both 10° and 24° VFs improved significantly following AG-PRP, and this central functional improvement may be due to a reduction in oedema following AG-PRP. Binocular standard driving VFs performed within 6 months of AG-PRP may actually reflect pre-existing VF abnormalities due to severe retinal ischaemia or non-viable retina at presentation, rather than direct functional loss from laser.

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Bilateral central serous chorioretinopathy in a patient with focal segmental glomerulosclerosis

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Purpose The central serous chorioretinopathy (CSCR) is a frequent ocular disease characterized by a serous detachment of the neurosensory retina at the posterior pole. This sporadic and self-finished disease can become chronic in some cases, resulting in a degeneration and atrophy of the underlying retinal pigment epithelium (RPE). The more severe cases of CSCR had been recognized in patients treated with high systemic corticosteroid doses and in haemodialysis patients.

Methods A 37-year-old-man was attended in emergency with history of several days of blurring of vision. He was being haemodialysis by chronic renal failure secondary to a focal segmental glomerulosclerosis. The patient presented a visual acuity of 0.4 in the OR and 0.8 in the OL, with an image of bilateral CSCR on the fundus examination that was confirmed by optical coherence tomography (OCT).

Results We reported the chronic evolution of the process until a kidney transplantation was done to the patient, reaching a spontaneous healing. Four and half months after the transplantation, evidences of a disappearance of sub-retinal fluid with a persistence of drusen-like retinal pigment epithelial alterations were found in the OCT-image.

Conclusion Immune-complex deposits similar to the glomerular deposits can be identified near the Bruch membrane in patients with glomerulonephritis, these deposits prove the RPE-barrier dysfunction and the appearance of disorders like macular drusens, sub-retinal fluid or choroidal neovascularization. The case highlights the importance of ophthalmological assessment in these patients to recognise sight-threatening complications.

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Internal limiting membrane surrounding idiopathic stage 4 macular hole contains bundles of actin microfilaments

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Purpose To study internal limiting membrane (ILM) specimens obtained from a case of stage 4 macular hole surgery with confocal microscopy and investigate the presence of actin microfilaments.

Methods A 68 year-old woman underwent 20-gauge transconjunctival sutureless vitrectomy for stage 4 idiopathic macular hole. Triamcinolone –acetone was used for visualizing the vitreous hyaloid. Following vitrectomy, the ILM was stained with Brilliant blue dye, then peeled off using an intraocular forceps. Two separate ILM specimens were obtained during surgery; the first around the macular hole and the second one disc diameter away from the macular hole up to the temporal vascular arcades and the optic disk. At the end of the surgery, air-gas (C3F8 16%) exchange was performed. The ILM specimens were studied immunohistochemically using confocal microscopy after labeling with antibodies to actin and fibronectin.

Results The ILM specimen around the macular hole was stained positive for both fibronectin and actin, whereas the ILM specimen away from the macular hole was stained positive only for fibronectin. Preoperative evaluation of the macular hole biomicroscopically and imaging with optical coherence tomography showed absence of any epiretinal membrane (ERM).

Conclusion ILM surrounding stage 4 macular hole contains bundles of actin microfilaments, suggesting the presence of cells with contractile properties even in the absence of associated ERM. These findings suggest that tangential traction may contribute to the pathogenesis of late stage macular hole development and/or enlargement and support removal of ILM in late stage macular hole surgery.

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Long-term visual prognosis in necrotising herpetic retinitis

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Purpose To determine the long-term ocular complications and visual outcome in patients with necrotising herpetic retinitis (NHR).

Methods 26 patients (33 eyes) with NHR diagnosed between June 1987 and January 2002 at Pitié-Salpêtrière Hospital, in Paris, France, were retrospectively reviewed. Demographic data, clinical presentation, viral identification (when possible), initial therapeutic management, long-term antiviral therapy, complications and final visual acuity were analyzed.

Results 16 patients (61.5%) were male, 10 (38.5%) were female. Age ranged from 11 to 67 years (mean 40.6 years). All patients were treated with intensive intravenous antiviral therapy, 9 (34.6%) with additional intravitreal ganciclovir injections. At last examination, 20 patients (76.9%) were still under prophylactic antiviral treatment. Complications were cataract (63.6%), retinal detachment (48.5%), recurrence (33.3%), macular edema (36.4%), optic atrophy (30.3%) and epimacular membrane (27.3%). Final visual acuity was below 20/200 in 15 eyes (45.5%). The minimal follow-up was 3 years.

Conclusion Despite aggressive antiviral therapy, visual prognosis remains guarded in patients with NHR. Retinal vasculopathy and optic disc alterations are major factors to consider in order to improve the visual outcome.



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Mechanics of a macular hole: formation and surgical closure

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Purpose To elucidate the mechanics of the formation and surgical closure of a macular hole.

Methods A parafoveal insertion of vitreous is examined in relation to its effect on the fovea. A force vector analysis is performed to understand the stages of a macular hole formation. A viscoelastic mechanical model is introduced to interpret the time constant of closure of the hole in terms of the physical properties of retina and the surrounding viscous fluid.

Results Macular hole develops due to a combination of a tangential (radially outward from the fovea) and perpendicular force components arising from the posterior hyaloid separation (insertion) close to the fovea. The perpendicular force leads to the anterior deformation of the fovea, and therefore in cysts and schisis ("cavitation") formation. The subsequent separation of the photoreceptors from the RPE is also a consequence of the same perpendicular force. The loss of contact with the RPE in the presence of a tangential force lowers the resistance to the radially outward deformation of the foveal tissue and eventual hole formation. The hole closure occurs due to the stored elastic energy of the retina. The relaxation of the fovea is slowed or prevented by the viscosity of the surrounding fluid. Therefore, the hole closure may occur as soon as a few hours or it may never occur depending on the residual fluid and its viscosity at the conclusion of the surgery. The contribution of ILM peeling is discussed.

Conclusion Macular hole forms as a consequence of both tangential and perpendicular components of the vitreous traction. The hole closure is promoted by the elasticity of the retina, and retarded by the viscosity of the residual fluid in the foveal region.

■ 464

Vitreous haemorrhage and retinal vasculitis as manifestations of cat-scratch disease

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Purpose To report a rare case of vitreous haemorrhage and retinal vasculitis secondary to cat scratch disease, caused by Bartonella henselae.

Methods A 42-year-old apparently healthy farmer presented with a 5-day history of blurred vision in his right eye. Right visual acuity was 20/25. Slit-lamp examination of the right eye revealed mild vitreous haemorrhage and diffuse retinal haemorrhages in the middle-peripheral retina. The left eye was unremarkable. Fluorescein angiography showed signs of retinal vasculitis in the right eye. The patient owned several cats, but had no history of cat bites or scratches.

Results A blood sample taken on the day of admission revealed the presence of IgM (titre=1:40) and IgG (titre=1:80) to B. henselae. Accordingly, the diagnosis of cat-scratch disease was made. The retinal vasculitis resolved after 8 days of therapy with oral azithromycin.

Conclusion Apart from neuroretinitis, the ocular complication associated with cat-scratch disease may include vitreous haemorrhage and retinal vasculitis.

■ 465

Syphilis, return of an old disease?

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Purpose Syphilis is a sexually transmitted infectious disease which can present with a large variety of symptoms. Recent rise in syphilis is described notably among homosexual men.

Methods We conducted a retrospective chart and patient database review of ocular syphilis cases in tertiary eye clinic. Main outcome measures: resolution of signs and symptoms of ocular syphilis, including changes in visual acuity.

Results In last five years five patients were treated for ocular syphilis (four in last two years). All patients were male, mean age 40, range 24-50 years. In all patients only one eye was involved, presented with visual decline. Clinical findings were: visual acuity 0,8 in one, 0,5 in one, and counting fingers in 3 patients; multifocal chorioiditis in 3 (with rhegmatogen retinal detachment in one), placoid chorioretinitis in one and optic neuropathy in one patient. The patients had positive serum fluorescent treponemal antibody absorbed tests and treponemal immobilization test. After the therapy visual acuity improved to 1,0 in four patients, but in one patient remained counting fingers.

Conclusion Syphilis has reemerged in the past few years and ocular findings, including chorioretinitis and optic neuropathy are important diagnostic features in the early diagnosis and management of this potentially fatal disease.

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