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Some reflections on corneal thickness

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The corneal thickness as an object for studies was recognized in the renaissance. A value of 1 mm, representing the maximally swollen human cornea, was reported. Optical in vivo measurements were done by Blix in 1880 reporting a thickness of about 0.5 mm, the value that we today know is correct. Blix lived in ‘the golden age of physiologic optics’. His interest was the contribution of the cornea to the optical refraction of the eye, and was thus the distance between the anterior and the posterior surface rather than the thickness of the cornea as such. A biomechanical interest in corneal thickness was initiated by the studies of tonometry, in particular Hans Goldmann’s development of appplanation tonometry. He predicted correctly that corneal thickness would influence the estimated pressure reading. Another physiological aspect of the cornea is its transparency. Earlier explanations by equal refractive index was revolutionized by the interference theory by David Maurice. Optical transparency required a regular fiber pattern, and thus a stabilized thickness and stromal hydration. This led to extensive interest in the permeability of the limiting layers, in particular the transport of fluid across the endothelium. The physiological concepts required a regulated or stabilized thickness. The thickness as such became interesting. The human cornea is thinner in the center than more peripherally and the central, presumably regulated central thickness (CCT) became a biometric and clinical study object. The exact individual value became of interest. Several optical and later ultrasonic principles were presented. Questions addressed were: Is CCT a life-long, age independent characteristics. Is CCT diagnostic for certain disease conditions (e.g. Macular dystrophy of Groenouw). Is CCT a useful clinical parameter to follow disease processes (e.g. progression in keratoconus or acute changes in graft rejections). Today refractive surgery has revived the interest in biomechanical and optical properties of the cornea. Modern computer technology allows for a description of the ‘thickness profile’ of the entire cornea. This gives us access to an overwhelming amount of data, and reopen many issues of the past. We must realize, however, that what we see is the pendulum swinging back to the problems of the last century. The machinery is smarter but many of the basic questions remain to be solved.
From Helmholtz to the scanning laser ophthalmoscope

150 years of ophthalmoscopy

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Purpose To give a brief overview on Helmholtz and the history of ophthalmoscopy.

Methods Literature survey.

Results Hermann von Helmholtz (1821-1894), although not an ophthalmologist but a physiologist, invented the ophthalmoscope, not yet 30 years old, and is thus one of the most important scientists in European ophthalmology. Yet Charles Babbage may have preceded Helmholtz. Unfortunately an “expert” gave him a negative critic and his work was only published in 1854, 3 years after Helmholtz’s monography: “Beschreibung einer Augenspiegels zur Untersuchung des Netzhaut im lebenden Auge”. Helmholtz’s discovery as well as the first international congress of Ophthalmology exactly 150 years ago, introduced the modern era of ophthalmology. Within a few years several modifications of Helmholtz’s ophthalmoscope were presented by Ruete (1852), Coccia (1853 and 1855), Anagnostakis (1854), Gillet de Grandmont (1859), Galezowski (1862), Beale (1869) and others. Marc-Antoine Giraud-Teulon (1861) designed the first binocular ophthalmoscope. The first coloured drawing of the fundus was published in Utrecht in 1853 (Van Tricht) and Fallon (1863) published the first atlas of ophthalmoscopy.

Conclusion Despite limitations due to poor illumination the introduction of ophthalmoscopy resulted in the discovery of a completely new field in ophthalmology.
Can uveal melanoma be conquered?

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The deadly natural history of uveal melanoma was fully described, unknowingly, in a well known English artist in 1792 and soon thereafter in an unknown Scottish woman around 1808. It became a well recognised entity much later, by 1868. Today, with the exception of being able to save the eye of their patient, ocular oncologists managing patients with uveal melanoma find themselves in essentially the same situation than their forebears: mortality rates have not noticeably decreased and metastatic melanoma continues to be the “hideous picture of disease” that it was 150 years ago. Metastatic melanoma is the single overwhelming cause of death in patients with uveal melanoma, and no consistently effective treatment is known for disseminated disease. One reason for this unhappy state of affairs is that patients formerly were dismissed after enucleation until they presented with advanced metastasis to an oncologist who did not recognise uveal melanoma as a disease very different from cutaneous melanoma. The advent of ocular oncology has led to rational early detection programs for subclinical metastasis, validated staging of metastatic disease, and first controlled clinical trials of managing metastases with therapies specifically aimed against this cancer. Basic research highlights uveal melanoma as a typically slowly growing, early metastasising cancer; and staging, grading and typing of primary tumours is leading to rational assignment of patients to follow-up and adjuvant treatment trials, which hopefully will improve their survival rate. The current understanding is that, by the time the eye becomes symptomatic, uveal melanomas prone to metastasis already have seeded micrometastases, which need to be kept under control if we are to eventually conquer this disease.
Keynote Lecture

« 2001

Delivery of Eye Care in Developing Countries - Role of Research

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Purpose To present the information and problems of global blindness and how research can help in addressing these problems.

Methods Blindness is a serious public health problem in developing countries. Most (almost 90 percent) of avoidable – preventable and treatable – blindness exists in the developing countries of the world. Through a variety of innovations, based on epidemiological and operations research, innovative models of eye care delivery have been developed to make eye care effective and cost-effective. The next step is large scale replication with appropriate local modifications. A challenge before the research community is to extend the scope of translational research from the traditional "laboratory to the clinic" mode to an enhanced version of "laboratory to clinic to community" mode.

Results The eye care delivery model should encompass all levels of care from the primary to advanced tertiary with clear delineation of functions at every level, referral linkages, without compromising on quality or equity and making programmes sustainable. While prevention strategies against trachoma and onchocerciasis are successful and tackling cataract problem is getting better, the more complex issues of glaucoma and diabetic retinopathy are looming large. Significant attention has to be paid to develop appropriate public health and clinical approaches to control blindness from these problems too if VISION 2020: The Right to Sight has to realize the aspiration of controlling global blindness to a significant degree by 2020.

Conclusion Global cooperation and collaboration may be the key to arrive at solutions in an accelerated manner given the environment of increasingly limited resources and competing demands.
2002

The retinal pigment epithelium, friend or foe?

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Purpose To review the physiology and pathophysiology of the retinal pigment epithelium, RPE, with focus on some of the work of myself and my wonderful collaborators and students during the last 20 years.

Methods In vitro studies of RPE preparations from frogs and of cultured human and porcine RPE cells. In vivo studies of experimental surgery in pigs using histopathology, immunohistochemistry and functional testing by multifocal electoretinography.

Results There are active fluid transport mechanisms in the RPE, which are capable of transporting water against an osmotic gradient. This has important bearings on the fundus appearance in states of macular oedema. RPE cell physiology is dependent on the surface on which they grow, and the maturity of the cells. Older RPE cells are more stress resistant than younger RPE cells. This has important bearings on studies where cultured RPE cells are used to assess toxicity of substances used in the clinic.

In vivo RPE cells are non-dividing under normal circumstances. Posterior injury to the epithelium induces proliferation of the peripheral, but not the posterior RPE. This indicates that a g zone of putative RPE stem cells might exist in the peripheral retina. The functional consequences of experimental posterior RPE injury can be assessed by multifocal electoretinography

Conclusion The RPE plays an important role in the normal physiology of the outer retina, as well as in the pathogenesis of retinal diseases. The development of animal models of RPE injury increase our understanding of the role of RPE in health and disease.
Antigen-presenting cells and the eye

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Antigen-presenting cells (APC) carry many different functions such as phagocytosis, antigen processing, antigen presentation. Depending on the nature of the APC, presentation of an antigen may lead to an active immune response, or lead to inhibition of the specific immune response. Some ocular subjects where APCs are of special interest are corneal immune responses, e.g. against viral infections, the development of ACAID (anterior chamber associated immune deviation), and tumor immunology. In the last case, a good response might help the patient survive, but the development of an inferior response might suppress any useful anti-tumor responses. These controversies will be discussed.
Keynote Lecture

New perspectives in retinal imaging: fundus autofluorescence and age-related macular degeneration

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Fundus Autofluorescence (FAF) imaging using confocal scanning laser ophthalmoscopy is a non-invasive method to accurately record the topographic distribution of RPE lipofuscin in the human eye in vivo. Excessive lipofuscin accumulation in the RPE is a common downstream pathogenetic pathway in various complex and monogenetic retinal diseases. Toxic compounds and molecular mechanisms of interference with normal cellular functions have been identified including the dominant fluorophore A2-E. Alterations in fundus autofluorescence (FAF) appearance in eyes with early and late age-related macular degeneration (AMD) can be striking. FAF patterns and distribution do not necessarily correlate with the features of interest in color or angiographic images of eyes with early or late AMD. In the prospective, multicenter FAM study distinct patterns of abnormal FAF were identified and classified in the junctional zone of geographic atrophy (GA). Areas of increased FAF outside GA were associated with variable degrees of loss of retinal sensitivity when tested with microperimetry which suggests a functional correlate of lipofuscin accumulation. Increased FAF preceded the development and enlargement of outer retinal atrophy associated with spread of absolute scotoma in eyes with AMD. Longitudinal examinations showed that the abnormal phenotypic FAF patterns serve as novel prognostic determinants which allows to distinguish fast vs. slow progressors. These findings are relevant and now used to design and carry out interventional trials with agents aimed at slowing down spread of atrophy, e.g. using visual cycle modulators to influence lipofuscinogenesis. Hereby FAF imaging also serves as a mean to accurately delineate and measure areas of GA over time in an automated fashion. A phenotype-genotype correlation was identified for a distinct FAF phenotype subset which was found to represent late-onset Stargardt macular dystrophy mimicking late-stage atrophic AMD. New imaging technologies were recently applied including simultaneous recordings of FAF images and high-resolution, spectral-domain optical coherence tomography (OCT) which allows to identify morphological correlates of abnormal FAF signals in optical biopsies.
Ophthalmic Research Lecture: Active subretinal implants in seven blind patients: outcome of a pilot study and further developments

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Posters

THURSDAY

- Posters 201 - 271, exhibited on Thursday .......... 16-34

FRIDAY

- Posters 301 - 370, exhibited on Friday .............. 35-52

SATURDAY

- Posters 401 - 473, exhibited on Saturday .......... 53-71
Poster session 1: Anatomy/Cell Biology - Cornea/Ocular Surface - Immunology/Microbiology - Molecular Biology/Genetics/Epidemiology - Vision Sciences/Electrophysiol./Physiol. Optic

201 / 2357
Characterization of efflux proteins in human corneal epithelial cells

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Purpose Corneal epithelium is the main barrier for absorption of drugs into intraocular tissues after topical administration and part of this barrier may be formed by efflux proteins which translocate molecules from the cell interior to the extracellular space. The aim of this study was to characterize the gene expression and the activity of the efflux transporters in the cell culture model of immortalized human corneal epithelial cells (HCE cells), in primary cell line (HCEPIc), and in the human corneal epithelium.

Methods The mRNA levels of MDR1, MRP1, MRP3, and BCRP were determined by the quantitative RT-PCR. Immunochemistry was used to study protein expression and localization of efflux transporters. Functionality of these proteins was assessed with calcein-AM efflux assay and by measuring the efflux of CDCE. Furthermore, bidirectional permeability of rhodamine 123 (Rh123) was studied.

Results The mRNA of MDR1 and MRP5 were detected in the human cornea and in both cell lines. These efflux proteins were found in the cell membranes of the human corneal epithelium. At mRNA level some efflux proteins were over-expressed in the HCE and the primary cell lines. Increased calcium retention and decreased CDCE efflux in the presence of inhibitors suggested efflux protein activity in both primary and HCE cells. Likewise, directionality in Rh123 permeability was diminished in the presence of verapamil in HCE model.

Conclusion Functionality of the efflux proteins was demonstrated in the human corneal epithelial cells. MRP1 and MRP5 may have important protecting role in corneal surface by transporting molecules out from the epithelial cells. It seems that the efflux activity in the HCE model differs from that of the corneal epithelium in vivo.

202 / 2456
Bio-available zinc in the outer segments of short wavelength cones

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Purpose Zinc has been postulated to play a role in both normal and degenerative processes in the retina. Often these processes require zinc that is tightly bound to enzymes. However, new evidences indicate that the presence of bio-available zinc is a better indicator for the involvement of zinc in retinal fusions. Using autometallography on sectioned tissues suggested that bio-available zinc is absent in photoreceptor outer segments. The purpose of this study was to re-examine this observation using a zinc selective fluorescence probe and freshly dissected, flat mounted retina.

Methods Following deep anaesthesia and decapitation, dark-adapted retina were immediately dissected from rat, goldfish and chicken eyes and flat mounted with the photoreceptor up. The retina were immediately labeled with the zinc-specific fluorescent probe ZPI (Neurobiotix, USA). Readily releasable zinc was visualized using fluorescent and confocal microscopy.

Results Only a subset of photoreceptor outer segments were labeled by ZPI in rat retina (≤0.5%). This labeling was localized to a subset of the cones. The cone localization of this labeling was confirmed by using goldfish retina where the cones are readily distinguishable from rods morphologically. To identify the subtype of zinc positive cones we labeled chicken retina where the characteristic oil droplets within the cones identify their sub-type. Here zinc appeared to be localized to short wavelength cone outer segments.

Conclusion This is the first demonstration that bio-available zinc is present in photoreceptor outer segments. The specific localization to short wavelength cones suggest that zinc plays a selective role in visual processing of short wavelength light.

203 / 2457
Metabolic stress as a contributor to physiological opening of TRP channels in blowfly (Calliphora vicina)

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Purpose TRP channels in blowflies can be reversibly activated by metabolic stress. Activation can be caused by hypoxia or mitochondrial uncoupling and results in membrane depolarization. The changes where traced as changes in extracellular ionic composition, photoreceptor membrane currents and changes in membrane potential. Idea of specific signaling pathway opening TRP channels was rejected with findings that impairment of PIP2 under lack of ATP results in activation of TRP channels. Even though the mechanism of metabolic stress TRP channel activation is elucidated, it remained unanswered what rate of metabolic stress is needed to open the channels and whether under physiological conditions photoreceptor cells encounter the rate of metabolic stress that can significantly contribute to opening of TRP channels.

Methods Extracellular concentrations of [K+]o, [Na+]o and [Ca2+]o were measured using the ion-selective microelectrodes. The redox states of respiratory pigments (flavoproteins and cytochromes) in the eyes of blowflies (Calliphora vicina) were measured with time-resolved absorption spectroscopy and use of principal components analysis. Anoxia was reached in about 21 min.

Results Changes in [K+]o, [Na+]o and [Ca2+]o appeared with latency of 8.06 ± 1.38 s, 10.38 ± 2.48 s and 13.11 ± 3.09 s, respectively (mean ± s.e.m., n=7). First detectable changes in redox state of cytochrome c respiratory pigment started at 1.79 ± 0.19 s and reached 39.4% ± 5.4% of the maximal reduction by the time changes in ionic composition were detectable.

Conclusion Our conclusion was that high degree of maximal redox state change is needed (39.4%) to open TRP channels, what is unlikely to happen under physiological conditions.

204 / 2458
Persistent pupillary membrane remnants attached to the anterior lens capsule

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Purpose To present an interesting case of bilateral persistent pupillary membrane remnants without visual acuity impairment. Persistent pupillary membrane remnants are an ocular 'congenital anomaly' and often affect both eyes.

Methods A 12 year old dark-haired boy presented on a routine examination with dense and wide pupillary membrane remnants attached to the iris collarette and the anterior lens capsule bilaterally. In OD the remnants covered almost the whole pupillary area however leaving some clear islands and among them a central one. In OS the remnants did not cover the central pupillary area.

Results Uncorrected visual acuity was surprisingly good (9/10 OD and 10/10 OS). Although auto-refraction was OD: +3.50 -2.25 X 180° and OS: -0.75 -0.25 X 170°, subjective refraction was OD: +1.25 X 90° proving 10/10 and Jaeger 1 and OS: plano providing 10/10 and Jaeger 1.

Conclusion The characteristic of the pupillary membrane remnants is the attachment to the iris collarette and this is the most important differential diagnostic feature from pupillary membranes of other origin. Surgical removal of these membranes or YAG laser membraneotomy is sometimes necessary in cases with severe visual impairment. The surprisingly good visual acuity in the present case is not suggestive of any attempt to remove these pupillary membrane remnants. Excellent near and distant visual acuity was preserved in the right eye due to a 'pinhole effect' of the central clear island which prevented the occurrence of amblyopia.
**205**

**Interleukin-1-Induced apoptosis of keratocytes: effect of biglycan**

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**Purpose** Biglycan is absent in the normal cornea, but UVR exposure leads to a significant expression of the biglycan gene in the rabbit cornea, an effect that decreases after healing is completed, indicating the involvement of biglycan in the corneal repair process. In the present study, we have investigated possible involvement of biglycan in the modulation of the survival of keratocytes.

**Methods** Keratocytes were grown either under serum free conditions to obtain quiescent keratocyte cell culture or in the presence of 10% fetal bovine serum to induce keratocyte transformation into myofibroblasts. Myofibroblastic phenotype was confirmed by immunocytochemistry with anti-alpha-smooth muscle actin antibodies. Cell death was induced in both cell cultures by interleukin-1 in the presence or absence of biglycan. Histone-associated DNA fragments were assayed by using a cell death detection ELISA.

**Results** Quantification of histone-associated DNA fragments by the cell death detection ELISA showed that biglycan strongly protected quiescent keratocytes from dying whereas it enhanced the death rate of transformed keratocytes. Apoptotic death rate was elevated after the addition of IL-1 in both keratocyte and myofibroblast cell cultures. Co-incubation with biglycan markedly reduced the number of apoptotic keratocytes but markedly increased the number of apoptotic myofibroblasts.

**Conclusion** IL-1 induces apoptosis of both quiescent and transformed keratocytes. However, biglycan has differential effect on apoptosis of these two cell types.

**206**

**Role of EGFR in corneal remodelling in diabetes**

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**Purpose** To examine the role of epidermal growth factor receptor (EGFR) signalling on ultrastructural organisation and remodelling of stromal corneal collagen ﬁbres (CF) and proteoglycans (PG) in the diabetic rat. 

**Methods** Diabetes was induced in female Wistar rats (n=5) by streptozotocin (STZ) injection (55mg/kg). Treatment with a selective inhibitor of EGFR tyrosine kinase, AG1478, was started on the same day as the induction of diabetes and administered every other day for four weeks. The corneas were fixed in 4% PFA at 4°C to analyse the collagen ﬁbrel diameter. To study the distribution of proteoglycans the corneas were fixed in 2.5% glutaraldehyde in sodium acetate buffer containing cuprocin blue. The analysis was performed on control and EGFR treated corneas.

**Results** In addition to a reduction in the epithelial thickness in the diabetic cornea, the median diameter and area fraction of CF in the stroma was decreased in diabetic rats compared to normal rats (p<0.001). In contrast, the median PG area and area fractions in diabetic rat corneas were signiﬁcantly increased (p<0.001) compared to normal. Treatment with AG1478 prevented diameter and area fraction changes in CF and PG and restored corneas to a normal appearance.

**Conclusion** These data show that the distribution of stromal CF and PG is altered in corneas after 4-weeks of diabetes. Furthermore, treatment with an inhibitor of EGFR signalling normalized these abnormalities. Thus our data suggest that the EGFR plays an important role in the development of diabetes-induced corneal remodelling.

**207**

**Ghrelin expression in the iris and ciliary epithelium of fetal and newborn rats**

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**Purpose** The presence of ghrelin was previously identiﬁed in the posterior epithelium of the iris and in the non-pigmented ciliary epithelium. Ghrelin is also a potent relaxator of the iris sphincter muscle.

**Methods** Sprague-Dawley rat fetuses were removed by cesarean section at 10.5, 11.5, 12.5, 13.5, 17.5, 20.5 dpc, and 15 day-old animals (P15) were euthanized. Specimens (hearts or eyes) were paraffin embedded and processed for in situ hybridization (ISH). Digoxigenin labelled ghrelin probe was synthesized from a 2371bp fragment of prepro-ghrelin.

**Results** Ghrelin’s mRNA was expressed in the posterior epithelium of the iris at 17.5, 20.5 dpc fetuses and P15 animals. Furthermore, in 20.5 dpc fetuses and P15 animals there was also ghrelin expression in the non-pigmented ciliary epithelium.

**Conclusion** Ghrelin is expressed during development of the rat’s eye from 17.5 dpc onward. Its expression is more evident in the posterior epithelium of the iris and ciliary epithelium. These ﬁndings could exert a new function of ghrelin in the development of the anterior segment of the eye.

**208**

**Crosstalk of Hsp70 molecular chaperone, lysosomes and proteasomes in macroautophagy-mediated proteolysis in human retinal pigment epithelial cells**

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**Purpose** Pathogenesis of AMD involves chronic oxidative stress, impaired degradation of membranous discs and accumulation of lysosomal lipidfucin in retinal pigment epithelial (RPE) cells. It has been estimated that a major part of cellular proteolysis occurs however in proteasomes. Prior to the proteolysis heat shock proteins tend to refold misfolded proteins and thus prevent the accumulation of protein aggregates. Connection of Hsp70, lysosomes and proteasomes was evaluated in the protein aggregation process in human RPE cells (ARPE-19).

**Methods** Cellular localization of Hsp70 was studied by immunofluorescence. Confocal and transmission electron microscopy were used to detect cellular organelles and morphological changes in ARPE-19 cells. Cell viability was analyzed by MTT assay. The modulations of Hsp70 levels were done by using siRNA and overexpression vector techniques.

**Results** Proteasome inhibitor caused formation of juxtanuclear protein aggregates. It also caused robust accumulation of Hsp70 protein which colocalized with the proteasome aggregates. The granulation of aggregates was clearly increased in response to proteasome inhibition and simultaneous hsp70 mRNA interference. We found that protein aggregation is an interim, a cause of proteasome inhibition led to autophagosome-mediated cleaning of cytoplasmic protein aggregates. The Hsp70 colocalization was observed in both primary lysosomal fractions and in autophagosomes.

**Conclusion** This study reveals that Hsp70, lysosomes and proteasomes are connected each other in macroautophagy – mediated proteolysis in human RPE cells.
Effects of UDP on ARPE cells: possible involvement of P2Y6 receptors

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Purpose: We have studied the effects of nucleotides (ATP, UTP and UDP) on human retinal pigment epithelial cells in relation to their expression of functional P2Y6 receptors.

Methods: ARPE cells were grown in culture and stimulated with ATP, UTP and UDP. Cell proliferation was analysed by BrdU incorporation. Calcium flux and cAMP were measured by fluorometry and radio-immunoassay. IL-8 production was investigated by semi-quantitative RT-PCR and ELISA. P2Y2, P2Y4 and P2Y6 expression was analysed by RT-PCR.

Results: UDP inhibited ARPE cell proliferation while ATP slightly stimulated it and UTP had no effect. This differential effect suggests the expression of other P2Y receptors than the already described P2Y2. Accordingly, we detected transcripts for the P2Y6 receptor, which is a UTP receptor. UDP induced a calcium flux like ATP's and UTP, but no cAMP production. All nucleotides stimulated IL-8 secretion.

Conclusion: Our results suggest that ARPE cells express functional P2Y2 receptors.

 ultrastructure of Femtosecond Laser trephination for keratoplasty

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Purpose: Femtosecond Laser are proposed as tools for trephination in keratoplasty. This novel technology is used to realize complex tissue dissection profiles that may potentially allow a reduced astigmatism, enhanced wound stability and more selective transplantation of tissue parts.

Methods: Using a 69kHz Intralase Femtosecond Laser (Intralase f660, Intralase Corp., CA) different trephination patterns were performed in ten porcine eyes. Corneoscleral specimen were subsequently cultured for five days before being processed for histological assessment. Corneal thickness before the procedure and after histological embedding was measured, the geometry of the dissection line was evaluated and the morphological characteristics of the cellular architecture along the lensation was analyzed at high magnification light microscopy.

Results: Straight and bevelled trephinations were realized with equal technical ease after explanation of the corneal curvature. The focus pattern lines within the corneal stroma presented as straight lines of 5-10 μm crossing the histoarchitecture of the collagen lamellae without deflection within of the corneal stroma. Sporadic matrix-removing negative spots were suggestive of cellular debris altered by the laser spots. Cell nuclei were also found scattered within the dissection line.

Conclusion: Tissue dissection for corneal grafting in complex bevelled patterns proved feasible with the Intralase-Device. Separation of corneal collagen lamellae occurred along the targeted line, leaving a linear cut without visible ablation of tissue nor discernable collateral damage to the surrounding tissue. The smoothness of the cut may allow, in clinical application, novel selective tissue transplantation, potentially minimizing interface haze.

Descemnet membrane endothelial keratoplasty using descemetorhexis and organ-cultured donor corneal tissue

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Purpose: To report results of two surgical procedures of endothelial keratoplasty.

Methods: Prospective, noncomparative study. Thirty three eyes of 38 patients with 21 corneal edema Fuchs dystrophy, 11 pseudophakic dystrophy, 4 decompensation of penetrating graft and 2 corneal edema after herpes endotheliitis all eyes, the recipient Descemnet membrane was excised using descemetorhexis through a 3.2 mm corneal incision. In 17 cases endothelial transplant was harvested by lamellar manual dissection. In 21 cases separation of endothelium was made performed with viscoelastic dissection. A temporary gas bubble was used for donor tissue adherence. Preoperative and postoperative best spectacle-corrected visual acuity (BCVA), manifest refraction, axial astigmatism, pachymetry and endothelial cell density (ECD) were evaluated 2 and 6 months after surgery.

Results: At the end of follow-up 37 grafts remained transparent and grafts were healed in good position. One eye required conversion to standard penetrating keratoplasty. After 2 months, BCVA in the group of fuchs dystrophy was 20/40 and 20/25 at 6 months. In the group of pseudophakic keratopathy, BCVA is 20/100 and 20/60 at 2 and 6 months respectively. The average MIR astigmatism was 1.3 D. The mean pachymetry is stable after 1 month at 540 μm. The average ECD at 2 months was 1500 cells/mm2 and 1300 cells/mm2 at 6 months.

Conclusion: This procedure, with its absence of corneal surface incisions and sutures, is a safe procedure that preserves the normal corneal topography, minimizes astigmatism and corneal power changes. This technique offers considerable advantages over penetrating keratoplasty in the treatment of endothelial dysfunction.

Corneoscleral transplantation for extensive infection of the cornea

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Purpose: Corneoscleral grafts have been used in the past for anterior segment reconstruction after extensive infection of the cornea. The aim of this study is to present the results of a series of ten consecutive anterior segment reconstruction using corneoscleral grafts.

Methods: We studied ten patients with widespread corneal infection and coagulative necrosis of the cornea. Common features of the surgical technique included a total limbal perimetry in an endeavor to save possible limbal stem cells. To mark the scleral surface and partially trephine this tissue we used trephines of 14-15 mm. After a careful haemostasis we entered the anterior chamber with a diamond knife. The entire corneal was removed and the donor corneoscleral graft was sutured into place on the scleral edge using interrupted 8.0 silk or 90 nylon sutures. The anterior chamber was filled with viscoelastic material and the conjunctiva was then sutured to cover the peripheral part of the graft. Postoperative steroid therapy was methylprednisolone (1mg/kg of body weight) and betamethasone 1% eye drops every two hours for 4 weeks and then every hour for a long term.

Results: After a mean time of 15 months after corneoscleral grafts four patients maintain a clear graft. Six patients could be defined as having typical rejection episodes with the corneas becoming opaque.

Conclusion: The technique of corneoscleral transplantation can salvage otherwise end stage eye disease, but the results are poor with respect to maintenance of vision. These patients need careful follow up because of potential complications especially graft rejection and recurrence of disease.
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NGF topical administration in dogs affected by spontaneous keratoconjunctivitis sicca

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Purpose To study the effect of Nerve Growth Factor (NGF) on spontaneous keratoconjunctivitis sicca (KCS) in dogs and to evaluate the potential therapeutic action of this molecule.

Methods The present study has been conducted on a sample of randomized dogs affected by spontaneous KCS. It consists of 6 cases (12 eyes), various ages (from 2 to 10 years old), different sexes (4 males - 2 females). All dogs showed different stages of mucous purulent filaments and redness; they were negative to fluorescein; Schirmer tear test I (STT I) on right eye was 5.8 ± 2.0 mm/1 min on left one was 6.0 ± 1.4 mm/1 min; cytology showed many inflammatory cells. The dogs were topically treated with purified murine NGF diluted in paraffin oil (volume: 30 microgram/ml) for 4 weeks (2 drops every 12 hours).

Results The result of these studies showed a significant improvement as evaluated by STT I. Thus, the right eye was 13.1 ± 2.4 mm/1 min (p < 0.05), left eye was 14.0 ± 2.4 mm/1 min (p < 0.05). Moreover, dogs treated with NGF displayed decreased purulent corneal secretion and redness and improvement of corneal transparency. Cytology analysis indicated a significant reduction in the number of corneal inflammatory cells.

Conclusion This report demonstrated that NGF is involved in the regulation of tears production, most probably by acting on tear-producing cells. NGF caused also a marked anti-inflammatory action of the dogs’ cornea. This result confirmed and extended previous evidences on human dry eye and previous findings suggesting a potential therapeutic utility of NGF in spontaneous KCS affecting dogs. Nevertheless, further clinical studies are necessary to assess the consistency of this clinic approach.

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Keratocyte repopulation in UVB-exposed thioltransferase knockout mice

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Purpose Thioltransferase is involved in cell protein homeostasis and DNA synthesis. It inhibits apoptosis and stimulates cell proliferation. Keratocyte repopulation after ultraviolet B (UVB) damage was studied in corneas of thioltransferase (-/-) mice.

Methods Six wild type mice and six thioltransferase (-/-) mice corneas were exposed at 300 nm UV radiation at a dose producing damage in the corneal stroma (8 kJ/m2). Animals were killed 3 and 7 days after exposure. Corneas were processed for light microscopy.

Results All corneas of wild type mice and thioltransferase (-/-) mice showed extensive damage 3 days after UVB exposure. Keratocytes disappeared throughout the entire thickness of the UVB-damaged central stroma. Corneal thickness was nearly doubled compared with non-treated control corneas. However, 7 days after UVB exposure corneas of wild type mice were almost completely repopulated by keratocytes, only superficial 1/3 of the stroma was still free of keratocytes. Corneal thickness was almost normal. Corneal stroma in the thioltransferase (-/-) mice 7 days after UV exposure was still not repopulated by keratocytes and the corneas were still very thick.

Conclusion The keratocyte repopulation in thioltransferase (-/-) mice is delayed. Thioltransferase seems to play an important role in the corneal wound healing and keratocyte repopulation after UVB induced damage.

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Corneal–conjunctival leukoplakia: histology and confocal microscopy

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Purpose To explore corneal–conjunctival leukoplakias using in vivo confocal microscopy and to compare images to histology and impression cytology specimens.

Methods Twenty-nine patients affected by corneal–conjunctival leukoplakia, after evaluation with slit lamp exam, were investigated with the Heidelberg Retina Tomograph II, Rostock Cornea Module. Subsequently a diagnostic excision or impression cytology of the cornea and/or conjunctiva was performed.

Results Histology identified seventeen Pterygia, six Pingueculas, two limbal stem cell deficiency (LSCD), one suture-related corneal vascularization, one cicatricial Pterygium and two corneal intraepithelial neoplasias (CIN). Confocal microscopy was compared to histopathologic sections and impression cytology specimens. In all patients typical white colour of lesions was attributable to a different degree of reactive hyperkeratosis. The main pathological features were visible on our confocal microscopy images: infiltration of conjunctival tissue surrounding lesions, vascularization, cellular morphology and activity, changes in extracellular matrix and modifications of limbar architecture.

Conclusion Confocal microscopy may be a useful aid for diagnosis and follow up in primitive and secondary corneal–conjunctival lesions. However the ability to provide high resolution, real-time images of the full thickness of the living human cornea and conjunctiva is strictly dependent on surface transparency and additional studies are required to establish exact correlations between confocal microscopy images and histopathology.

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Use of sodium hyaluronate “doughnut” in corneal glueing

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**Purpose**

To highlight a surgical technique using sodium hyaluronate and tissue glue in the repair of corneal holes.

**Methods**

Case Report

**Results**

An 86-year-old man was referred to clinic with a cataract. The other eye was normal after cataract surgery. When he was seen in clinic it was noted that actually he had a small hole in the cornea at the 5 o’clock position off axis. Visual acuity was 6/60. The iris was incarcerated and there was marked inflammation with posterior synechiae and cataract. The hole was felt too be small enough to attempt closure with tissue glue. To perform this a small ‘doughnut’ of sodium hyaluronate was placed around the hole. A very important step was to ask the gentleman to look up so that the ‘doughnut’ would not slide off. The glue was then carefully placed within the ‘doughnut’. When felt to be dry a bandage lens was placed over the eye. The cataract was also removed at the same time. The cornea healed very quickly and within 10 days post op was half of normal thickness. The gentleman now has 6/18 vision and a quiet eye with normal AC and IOP.

**Conclusion**

The use of tissue glue is widespread, it was first documented in use by midwives and then by medics in Vietnam. Their use in corneal perforations is also well documented. The problem however with their use is that the glue can tend to ‘stick’ to everything and cause complications. This is a marked problem with use on cornea due to the wet nature of the eye. Here we highlight a method whereby sodium hyaluronate is used to create a ‘doughnut’ to limit the spread of the glue and localise it to the area of need. What is also important to note is asking the patient to position their eye accordingly before placing the glue to achieve maximum effect as highlighted in other cases.

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Development of an image analysis device to measure transparency, folding and gerontoxon of organ cultured corneas

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**Purpose**

To develop an image analysis device devoted to the measurement of donor corneal transparency (T), folding (F) and clear corneal diameter excluding gerontoxon (G) during organ culture

**Methods**

High res digital images of a retroilluminated test chart comprising parallel lines viewed through the cornea were analysed using a dedicated software. T (%) was a ratio of local contrast of the test chart and F (%) was a ratio of mean of line profiles, each measured with and without the cornea. Area of clear cornea outside gerontoxon was calculated from a circle delineated by the observer. On each image, 3 independent experts classified T, F and G according to 3-level score (+, 0 and -) which served as reference. 179 human corneas (fresh, stored and deswelled) were consecutively assessed to include a broad spectrum of T and F.

**Results**

The device was able to discriminate between the 3 classes of T, F and G established by the experts. T was 20.4±36.8 and 51±10% respectively for transparency deemed poor, average and excellent (P<0.01). F was 29.18, 22.6 and 17.06 respectively for folding deemed high, average and minimal (P<0.01). Diameter of clear central cornea was 7.7±1.1, 8.9±0.8, 10.4±0.6 mm respectively for gerontoxon deemed severe, moderate and absent (P<0.01).

**Conclusion**

This simple and original device provides reliable quantitative objective measures of T and G. It will help standardising quality assessment of corneas among eye banks. A trial designed to assess the ability of the device to select corneas before delivery in the setting of the routine practice of our cornea bank is ongoing. Grants : Etabl Français des Greffes 2004 and PHRC/AC/01, 2007

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Counting strategies for endothelial assessment of organ cultured corneas using image analysis: comparison of border versus center method

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**Purpose**

To compare 2 cell count strategies, border and center methods for endothelial assessment during corneal storage.

**Methods**

7 observers determined the ECD, CV of cell area, and % hexagonality of 30 organ cultured corneas by the border (contour detection and manual retouch) and center method (indicating cell centers) using Sambucam analyzer. Interobs variability for ECD and agreement between the 2 methods was determined. The accuracy of the center method was verified on 10 standard phototographic mosaics (called keratotypes) by border (ref method) and center methods, first as fast and next as precise as possible (fast and slow modes respectively) and the time noted.

**Results**

For stored corneas, interobserver variability was: +0.6±0.5% (for border method and +0.7±0.5% for center method). ECD mean SD(range) (median) = [2948±565(644-3780)] (3081) and [2961±588(1736-3914)] (3017) respectively and showed excellent correlation (Pearson coefficient r=0.998, p<0.001). The center method underestimated the CV by a mean 2.7±0.5% (3.1-4.7%) and overestimated the hexagonality by a mean 2.5±0.5% (1.4-4.7%) (p<0.001 for both).

**Conclusion**

The center method gives accurate and reproducible ECD and is comparable to the border method (reference). However, morphometric evaluation is not fully reliable. Given that center method is easier to use in poor quality images, we recommend its use only for these cases. Grant: Etablissement Français des Greffes 2004

**220**

Ocular surface in dry eye patients is improved after one month therapy with Systane

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**Purpose**

Systane® ‘Lubricating Eye Drops’ [Alcon Forth Worth, Inc.] is designed to provide relief from dry eye by means of longer retention time on the ocular surface, based on the combination of the presence of PEG400, PG and HP-Guar (gelling hydroxypropyl-Guar) which is believed to mimic the mucin layer of tear film. The effect of Systane was evaluated in an open trial on subjects with moderate symptoms.

**Methods**

A total of 50 subjects were enrolled (age 57.6±13.5; 40 women, 10 men); inclusion criteria were based on a tear break-up time (TBUT) < 10 sec without corneal epithelial staining. Six symptoms of ocular irritation were rated on a four-point scale. Conjunctival hyperemia was graded. Patients were instructed to instill Systane four times a day for four weeks. Satisfaction with the product was rated at the end of the study. Media of results in both eyes was considered for statistical evaluation; the Wilcoxon test for paired data was applied; p<0.05 was considered as statistically significant.

**Results**

All patients regularly instilled Systane and completed the follow-up, results are expressed baseline vs endpoint. Significant reduction was demonstrated for ocular irritation symptom scores (1.44 vs 0.94, p<0.0001) while improvement was shown for patient’s satisfaction score (3.41 vs 3.94, p<0.0001) and TBUT (6.59 vs 8.5±1.5 sec, p<0.0001). A significantly increased proportion of patients with normal conjunctival injection (10 vs 22, results in %, p<0.01) was observed after one month follow up.

**Conclusion**

Systane proved effective in reducing the symptoms of dry eye and it was overall well accepted. This finding is likely to be related with improved stability of tear film, due to the protective viscous layer bound to the corneal surface.

**Commercial interest**
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**Changes in the α1 - α6 collagen IV chains in the corneas of posterior polymorphous corneal dystrophy patients**

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**Purpose**
To determine the changes in the expression and localization of the α1 - α6 collagen IV chains in Czech patients with posterior polymorphous corneal dystrophy (PPCD).

**Methods**
12 corneal buttons from 18 PPCD patients, as well as 8 corneal buttons obtained from healthy donors, were used. The tissues were snap-frozen in liquid nitrogen and stored at ~70°C until processed. Indirect enzymatic immunohistochemistry was performed using antibodies against the α1 - α6 collagen IV chains. The intensity of the signal was graded under a light microscope using a scale: +, +, ++, moderate, and +++ intense positivity. The localization of the signal was examined in all corneal layers, especially in the basilar membrane (BM), stroma and Descemet's membrane (DM).

**Results**
Staining for the α1 and α2 collagen IV chains was increased in DM of PPCD corneas and was shifted from the stromal side (in control tissue) to the endothelial side (in PPCD patients) of DM. We detected a more intense staining of α1 and α6 collagen IV chains in the posterior stroma of PPCD patients. We detected α1 and α2 collagen IV chains in BM of corneas of PPCD patients. Staining for the α3 - α6 collagen IV chains did not differ significantly between control and PPCD specimens.

**Conclusion**
The collagen IV chains show great heterogeneity in their localization in distinct corneal layers. The α1 and α2 collagen IV chains accumulate in DM, the stroma and the BM of PPCD patients. The increased expression of the α1 and α2 collagen IV chains and the change in their localization can contribute to the increased endothelial proliferative capacity observed in PPCD patients.

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**4α-PDD induces Ca2+ influx in human corneal epithelial cells by activating TRPV4 channels**

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**Purpose**
Transient receptor potential (TRP) isoform expression is evident in human corneal epithelial cells (HCEC-SV40). However, their role in maintaining corneal epithelial homeostasis is not fully understood. We probes for gene and protein expression as well as functional activity of the vanilloid subtype, TRPV4, in immortalized HCEC-SV40 since they elicited Ca2+ dependent regulatory volume decrease (RVD) responses during exposure to a hypotonic challenge.

**Methods**
RT-PCR and Western blotting analyses identified TRPV4 gene and protein expression. Functional activity was assessed based on determining whether the TRPV4 selective agonist, 4α-PDD, induced transients increases in intracellular Ca2+-concentration.

**Results**
Single cell fluorescence imaging results showed that 4α-PDD (3μM) increased intracellular Ca2+ concentration. The fus2 fluorescence ratio (F340/F380) was 0.39 ± 0.0378 in the resting state (n = 5). After application of 4α-PDD it increased to 0.904 ± 0.1436 (n = 5, p = 3.72077<10−5). This increase was abolished by the TRPV channel blocker ruthenium red or by Ca2+-free Ringer's medium.

**Conclusion**
In conclusion, functional TRPV4 expression in HCEC-SV40 4α-PDD expression may provide an osmoreceptor role to induce RVD behavior during exposure to a hypotonic challenge since this response is mediated through intracellular Ca2+ transients. Supported in part DFG PI 150/14-1 and NIH, EY04795. CR: none

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**Reconstitution of conjunctival epithelium under the influence of mesenchymal stem cells on a rabbit model of symblepharon**

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**Purpose**
Conjunctival fibrosis pathologies are hard to treat and liable to be recurrent. We worked out a project of cellular therapy by means of mesenchymal stem cells from the bone marrow so as to improve the therapeutic results.

**Methods**
We created an original model of symblepharon on the rabbit. We reconstructed the fornix thanks to an amniotic membrane covered, either with mesenchymal stem cells (MA/ASC), or conjunctival fibroblasts (MA/Fibro). Or with a single amniotic membrane (MA).

**Results**
The model of symblepharon presents a loss of anatomy with an average depth reduction of 60 % and a loss of function with goblet cells disappearance. After reconstruction, we had an anatomical restoration in all the operated eyes with a depth close to normal 10 ± 2, 11 ± 3 ET 9 ± 2 mm respectively in the MA/ASC, MA and MA/fibroblasts group. The difference between the groups is not statistically significant (p=0.65).The analysis of the conjunctival phenotype by immunohistochemistry MUC5ac shows a statistically higher density (p<0.05) of goblet cells in the MA/ASC 109 ± 25 group, than in the MA 32 ± 10 and MA/fibroblasts 10 ± 5 cells/mm2 group.Local inflammation estimated by histological analysis is lower and similar in the group MA/ ASC and MA with a average of 500 ± 20 inflammatory cells/mm in the fornical area than in the group MA/fibroblasts with an average of 1162 ± 50 cells/mm.

**Conclusion**
We demonstrated the feasibility of a reliable and reproducible animal model of conjunctival symblepharon. The anatomy is restored in the 3 groups but the presence of ASC allows us to find a conjunctival phenotype close to normal partly thanks to a decrease of the local inflammation.

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**Repair of corneal endothelium after mechanical damage under tissue culture and hypothermic conditions**

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**Purpose**
To evaluate the reparative capacity of corneas with mechanically induced endothelial damage stored under tissue culture (TC) or hypothermic conditions.

**Methods**
8 pairs of human corneas with similar qualitative and quantitative endothelial parameters were used. An area of 0.8 mm2 in the central endothelium was damaged by a flat metal tool. The paired corneas were stored under TC (E-MEM, 31°C) or hypothermic (Optisol, 4°C) conditions. The endothelial cell density (ECD), and the percentage of dead cells were assessed using a Luci computer analysis system before and after damage and on days 7, 14 and 21 following 12 hours of deswelling in a medium containing 5% dextran.

**Results**
The mean ECD before damage was 2769 ± 394/mm2. Immediately after the damage, a Descemet's membrane denuded and dead cells were observed in the injured area. After 7 days of culture, polymegathism and pleomorphism were observed in the central cornea, instead of little damage in the pericentral area. After 14 days, no damage or dead cells were observed in the injured area. ECD was 2229 ± 470/mm2 and 2156 ± 417/mm2 after 7 and 14 days of TC storage, respectively. A lesion with dead cells was observed after 7 and 14 days of hypothermic storage. The endothelium was completely repaired at day 21 in TC, while in hypothermy the corneas were cloudy and in poor condition.

**Conclusion**
The reparative capacity of the cornea is maintained under TC but not under hypothermic conditions. TC is therefore “the method of choice” for the storage of corneas with higher number of dead cells.
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**Effect of mitomycin C on epithelial hyperplasia after laser-assisted subepithelial keratectomy (LASEK)**

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**Purpose** To study the effects of mitomycin C (MMC) on the corneal regrowth after laser-assisted subepithelial keratectomy (LASEK).

**Methods** We performed a prospective, observer-masked study of 64 consecutive eyes (64 patients) scheduled to undergo LASEK to correct myopia. The patients were divided into two age-matched groups. 32 eyes in which the ablation depth was ≤50 µm were included in group 1 and received no MMC. 32 eyes in which the ablation depth exceeded 50 µm were included in group 2 and were treated with intraoperative MMC 0.02% for 30 seconds over the ablated zone. A masked observer measured the corneal thickness 1 and 3 months postoperatively. We compared the increase in central corneal thickness (CCT) during the postoperative period between both groups, assuming that this increment would correspond mainly to the epithelial hyperplasia that occurs after surface ablation.

**Results** The mean patient age was 31.5±4.6 years and 31.6±8.7 years in groups 1 and 2, respectively (P=0.9). Group 1 showed a mean CCT of 440±41.3 µm one month after surgery and 450.3±43.5 µm three months after surgery (P=0.04). CCT in group 2 was 399.7±31.2 µm one month and 407.9±32.6 µm three months after surgery (P=0.006). The difference between the CCT increments between both groups was not statistically significant (P=0.06).

**Conclusion** A single intraoperative application of MMC 0.02% during 30 seconds after laser surface ablation did not seem to cause a substantial change in the postoperative corneal thickening after LASEK.

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**Changes in corneal surface aberrations after myopic INTRALASIK and LASEK: Comparison between Technolas and Esiris**

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**Purpose** To compare high order corneal aberrations (HOCA) after myopic INTRALASIK and LASEK, using two different excimer lasers

**Methods** This prospective study included 237 eyes of consecutive patients divided into 4 groups: group 1 LASEK with Technolas (LT) 61 eyes, group 2 LASEK with ESIRIS (LE) 73 eyes, group 3 INTRALASIK with Technolas (IT) 61 eyes, group 4 INTRALASIK with Esiris (IE) 42 eyes.Uncorrected visual acuity (UCVA), best corrected visual acuity (BCVA) and manifest refraction were measured, in groups, before and 1 month after surgeries. The topography data were used to calculate HOCA with 6 mm pupil size, before and 3 months after surgery.

**Results** There were no significant differences concerning visual results 3 months after surgery in none of the 4 groups. However, there was an increase in total RMS and high order in patients treated with Esiris Laser. The aberrations with INTRALASIK (p=0.01) were higher. With Technolas laser, there were significant differences in total RMS, spherical and coma aberrations. In this case, aberrations after LASEK (p=0.01) were higher.

**Conclusion** Our results suggest that both excimer lasers produce an increase in HOCA with equal visual results. Higher HOCA were found after INTRALASIK with Esiris and LASEK with Technolas.

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**Strong expression of matrix metalloproteinases in the melted corneas of rheumatoid arthritis patients**

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**Purpose** To compare the expression and activity of matrix metalloproteinases (MMP) 1, 2, 3, 7, 8 and 9 in human corneal grafts obtained during penetrating keratoplasty for corneal melting with their expression in normal human corneas.

**Methods** Ten control corneal buttons and six melted corneas were used. All patients suffered from rheumatoid arthritis (RA); in three of them RA was associated with Sjögren’s syndrome, while two suffered from peripheral ulcerative keratitis and one from keratoconjunctivitis sicca. MMP 1, 2, 3, 7, 8 and 9 were detected using indirect enzyme immunohistochemistry. The activities of MMP 2 and 9 were examined in 10 control and 4 melted specimens by gelatin zymography.

**Results** Weak staining for MMP 1 and 2 and very weak staining for MMP 8 were detected in normal corneal epithelium. Moderate MMP 1, 2 and 9 staining and weak staining for MMP 3, 7 and 8 were observed in corneal epithelial fragments and in the anterior stroma of all melted corneas. Weak staining for MMP 1 and 2 was detected in the posterior stroma of the melted corneas. A strong presence of the zymogen and a weak presence of the active forms of MMP 7 were detected in all control corneas. Only the active form of MMP 2 was observed in three of four melted corneas, while in one of them both forms of this enzyme were present. The presence of the zymogen and the active form of MMP 9 was extremely strong in all tested melted corneas.

**Conclusion** The markedly increased expression of MMP 1, 2 and 9 and the presence of the active forms of MMP 2 and 9 in melted corneal grafts suggest that these enzymes may be responsible for the destruction of the extracellular matrix seen in grafts undergoing corneal melting.
Corneal backscatter after penetrating keratoplasty

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Purpose To determine changes in corneal backscatter in clear grafts after penetrating keratoplasty (PK) and in grafts that had failed from late endothelial failure (LEF).

Methods Sixty-one grafts, deemed clear by slit-lamp examination, in 46 patients and 12 grafts with LEF in 11 patients were examined at 1 to 30 years after PK. Backscatter was measured from the anterior, middle and posterior thirds of the cornea from a digitized image of a high magnification slit-beam through the center of the cornea. Clear grafts were compared to grafts with LEF and to 41 normal (unoperated) corneas of 41 subjects by using unpaired t-tests. Photopic low contrast visual acuity (LCVA) was measured in 32 of the clear grafts. Generalized estimating equation models were used to account for correlation between fellow eyes of the same patient.

Results Backscatter was increased by 80%, 49% and 34% in grafts with LEF compared to clear grafts in the anterior (P<0.001), middle (P<0.001) and posterior (P<0.001) thirds of the cornea, respectively. Backscatter was increased by 16%, 18% and 29% in clear grafts compared to normal corneas in the anterior (P<0.001), middle (P<0.001) and posterior (P<0.001) thirds of the cornea, respectively. Backscatter from clear grafts correlated with time after keratoplasty in the anterior (r=0.23, P=0.0055, middle (r=0.21, P=0.0006) and posterior (r=0.32, P=0.003) thirds of the cornea. LCVA correlated with backscatter in the anterior (r=-0.63, P<0.001), middle (r=-0.56, P<0.001) and posterior (r=-0.51, P<0.001) thirds of the cornea.

Conclusion Backscatter from penetrating corneal grafts is higher than normal, and substantially higher in grafts with LEF. Backscatter increases with time after keratoplasty, and increased backscatter is associated with decreased LCVA.

Corneal hysteresis in patients with corneal pathologies

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Purpose To investigate the corneal hysteresis (CH), the corneal resistance factor (CRF) and the corneal compensated intraocular pressure (IOPc) of subjects with normal corneas and with corneal pathologies with the Ocular Response Analyzer (ORA).

Methods IOP was measured using Goldmann application tonometry (GAT), dynamic contour tonometry (DCT) and ORA in 102 subjects with normal corneas and in patients with keratoconus (KC, n=32), Fuchs endothelial corneal dystrophy (FD, n=34) and penetrating keratoplasty (KP, n=50). Additionally central corneal thickness (CCT) and CH were quantified.

Results CH and CRF were significantly reduced in all groups with corneal pathologies in comparison to the normal group (CH: 7.7 (KC), 8.9 (KP), 7.3 (FD) versus 10.3 (N). CRF: 6.4 (KC), 9.3 (KP), 8.2 (FD) versus 10.8 (N) mmHg). High significant differences were also observed in mean IOP between the control group and the groups with corneal pathologies. There was a significant correlation between CH and CCT for normal corneas and corneas with KC and KP.

Conclusion CH and CRF are significantly decreased in patients with KC, FD and KP compared to subjects with normal corneas. Patients with corneal pathologies showed a substantial variability of mean IOP values evaluated by using different methods of IOP measurement in comparison to subjects with normal corneas suggesting that corneal properties strongly influence the validity of IOP measurements.

Effect of Mitomycin-C on the conjunctival goblet cell density after lasik

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Purpose To study the effect of mitomycin C (MMC) on the conjunctival epithelium after LASIK ablation.

Methods The present study comprised 18 patients scheduled for LASIK to correct myopia. The patients were divided into 2 groups of 9 patients each. In Group 1, the flap was performed with a mechanical microkeratome (M2, Moria), whereas in Group 2 a femtosecond laser (IntraLase) was used. For each patient topical MMC 0.02% was applied on the right eye with a Merocel sponge for 60 seconds, over the bulbar superior conjunctiva covering an area of 64 mm2 before the surgery. Conjunctival goblet cell density was studied by impression cytology pre-operatively, and at one, two and three months after surgery.

Results Analysis by impression cytology of the ocular surface showed that preoperative goblet cell density was not significantly different between the eyes with and without MMC (G1: P=0.81 and G2: P=0.50). 1 month after LASIK surgery there was a significant decrease in the goblet cell density in both groups (G1: P<0.008 and G2: P=0.022). However, there were no statistical differences in the goblet cell density between the right and left eye, at any time after the surgery, in any of the two groups (G1: P=0.62 and G2: P=0.08).

Conclusion Topical MMC 0.02% for 60 seconds over the bulbar conjunctiva before the LASIK surgery does not seem to affect the conjunctival goblet cell density in this preliminary study.
# 233 / 2216
Epidemiology of severe uveitis in France: a prospective multicentre study

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**Purpose** To determine the distribution and characteristics of severe uveitis, referred to university referral centres (URC) in France during a 3-month period.

**Methods** All new cases of uveitis referred between January and April 2006, to 31 specialized URC, for diagnostic or therapeutic management were prospectively reviewed. Characteristics of uveitis inflammation were reported at the end of the first examination on a questionnaire.

**Results** 527 patients were finally included. Median age was 44.1 (range 1.95) and the sex ratio was 1. The majority of patients (66.92%) were referred by their ophthalmologist and no previous episode of uveitis was noted in 67.72% of cases. Acute-onset uveitis was present in 60.22% of cases. Anterior uveitis was the most common type of presentation (54.7%) followed by panuveitis (21.1%). Complications at referral included a visual loss < 20/100 (20%), posterior synechiae (21.6%), secondary glaucoma (15.3%), cataract (7%), macular edema (7.8%) and retinal neovascularisation (1.7%). An infectious etiology was suspected in 27.8% of cases. Among these patients, a viral infection was suspected in 11.6% of cases followed by toxoplasmic chorioretinitis in 10.2% of cases. A non-infectious etiology was proposed in 46% of cases, including B27-like uveitis in 15.5% of cases.

**Conclusion** Even though classical forms of acute anterior uveitis are still referred to tertiary eye care centres, severe presentations of posterior or panuveitis should be managed properly. Initial ophthalmological examination is a key step for the diagnostic and therapeutic management of these patients. An infectious etiology may be easily suspected and should be excluded before the further use of corticosteroids or immunosuppressive drugs.

# 234 / 2217
Intermedial uveitis: clinical course in ten years follow up

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**Purpose** Intermedial uveitis (IU) is a chronic ocular disorder of unknown origin with frequent development of macular edema. The long term course of IU is not yet known and is a subject of this study

**Methods** Retrospective study of 28 patients with IU (12 males, 16 females; 49 affected eyes) in whom a follow up of at least 10 years starting with the onset of IU was available.

**Results** The average age at the onset of IU was 37. Five patients had an associated systemic disease (2 sarcoidosis and 3 multiple sclerosis). At onset bilateral involvement was observed in 20/28 (71%) patients. Visual acuity (VA) of more than 0.8 was noted in 19/48 (39%) affected eyes, and VA of less than 0.1 in 2/48 (4%) affected eyes. Systematic treatment with immunosuppressant drugs was required in 7/28 cases (25%) and 13 eyes underwent one or more surgical procedures (cataract surgery in 10 eyes, pars plana vitrectomy in 2 eyes, scleral buckling in 1 eye). At ten year follow up VA of more than 0.8 was noted in 22/49 (45%) affected eyes, and VA of less than 0.1 in 10; 49 (8%) affected eyes. The causes of visual loss included predominantly cataract, macular edema and vitreous opacities. Moreover, during the ten-year follow up intraocular inflammation diminished in 10/28 (36%) patients. The mean time to remission was 5-4 years (range 2-12 years). During the follow up, systemic disease sarcoidosis and multiple sclerosis manifested in two additional patients.

**Conclusion** The main complication of IU consisted of cataract, followed by macular edema and vitreous opacities. Within ten-year of follow up one third of patient with IU achieved the long term remission of the intraocular inflammation.

# 235
The effect of post-operative antibiotics on the ocular surface bacterial flora in cataract patients

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**Purpose** To study the nature of the preoperative bacterial conjunctival flora using real-time PCR and to assess whether there was any alteration in the flora due to phacoemulsification and a three week course of combination Tobermacyn and dexamethasone drops.

**Methods** Thirty patients undergoing routine cataract surgery were recruited to a prospective study. Posterior lid margin and inferior conjunctival swab samples were taken preoperatively from both the eye to be operated upon and the adjacent eye as a control. These swabs were analyzed by real-time PCR for a range of Gram positive and Gram negative bacteria recognised to often reside upon the ocular surface. Repeated sampling and analysis was performed three weeks postoperatively while patients were taking topical drops.

**Results** Staphylococcus epidermidis was the only bacteria demonstrated using this technique. Postoperatively a statistically significant increase was noted in the level of bacteria in the treated eyes (p<0.001) with 72% of operated eyes having higher bacterial levels than preoperatively. Overall the control eyes demonstrated higher levels of S epidermidis postoperatively compared to preoperative eyes p<0.05.

**Conclusion** S. epidermidis is one of the most common causes of endophthalmitis. Postoperative cataract drops are commonly used to reduce inflammation and as prophylaxis against endophthalmitis. This study questions the value of some commonly used prophylactic antibiotic preparations. It also demonstrates the potential for use of real-time PCR for rapid preoperative assessment of the conjunctival microbial flora in high-risk cases.

# 236
Pathogen or commensal: a PCR based study of ocular surface bacteria in normal and dry eyes

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**Purpose** To determine the ocular surface bacteria in normal and dry eye subjects and investigate whether there was any association between the bacterial population and goblet cell density (GCD).

**Methods** Fifty-seven normal and 34 dry eye subjects were recruited. Conventional culture, 16S rDNA PCR and DNA sequencing were used to identify bacteria. The association between reduced GCD and bacterial numbers in a sub-group of 27 subjects was assessed. Conjunctival impression cytology samples were stained with PAS and GCD graded as follows: Grade 1, >30 goblet cells/4 high power fields, (2), 15-30 GC/4HPF; (3), 5-15 GC/4HPF, (4), <5 GC/4HPF. Grades 3 and 4 indicated reduced GCD.

**Results** The majority of cultured bacteria were coagulase-negative Staphylococci, while molecular methods demonstrated additional atypical bacillary species. Bacterial levels were higher overall in subjects displaying reduced GCD compared to normals. A statistically significant difference (P = 0.005) was noted between mean bacterial counts in IC Grade 4 samples of dry eye subjects (135 cfu/swab) and normals (5 cfu/swab).

**Conclusion** Molecular analysis revealed a diverse ocular surface bacterial population with identification of potentially pathogenic bacteria presenting a diagnostic dilemma. A trend of increasing bacterial numbers with reduced GCD was observed and studies are ongoing to investigate whether bacterial colonization of the ocular surface may alter the number and function of goblet cells. The clinical relevance of such results and whether they should prompt intervention therapy is not yet fully determined. A fuller definition of the normal ocular flora is needed to determine bacteria implicated in ocular surface disease.
Incidence of endophthalmitis after cataract surgery: standard diagnosis versus molecular diagnosis

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Purpose New techniques as Molecular Biology (PCR) could reveal new data about the incidence of endophthalmitis after cataract surgery. The aim of this work is to know the real incidence of endophthalmitis when we use both methods as diagnosis of endophthalmitis.

Methods We have collected 72 samples from 57 patients showing clinical signs of endophthalmitis. These samples were taken from year 2000 to 2006, both included. Ocular samples were taken for microbiological study, including classical culture, Gram and Giemsa stains and PCR. All the samples obtained from the 17 patients were negative by the three methods used and all patients improved with steroid therapy. In total 53 aqueous and 19 vitreous samples were collected.

Results The results were positive using stains in 27% of the aqueous samples (AS) and 53% of the vitreous samples (VS), but without identifying the species in any samples. Classical culture could detect the pathogen in 53% of the AS and in 89% of the VS. The PCR was positive for fungal or bacterial primers in 85% of the AS and in 95% of the VS from the patients with infectious endophthalmitis. We have ascertained the incidence of endophthalmitis in our centre after cataract surgery, between years 2000 to 2006. If we only take into account the cases which were positive by culture the incidence is 0.14%. However, if we test the samples by molecular methods the incidence is greater due to the fact that sensitivity is higher. In this case the incidence is 0.19%.

Conclusion Application of PCR and molecular methods can help us to discover new pathogens involved in ocular infections. The higher sensitivity of molecular methods may reveal that the incidence of endophthalmitis is higher than previously suspected.

Antimicrobial treatment of presumed ocular tuberculosis

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Purpose Uveitis secondary to a tuberculosis is rarely reported, even in a tertiary care center. The prevalence of tuberculosis is low in Western Europe and its microbiological identification is difficult. However, anti tuberculostatic treatment may be useful when the diagnosis of tuberculosis is presumed.

Methods The clinical records of patients with suspected tuberculosis uveitis referred to the Ophthalmology Department of the Grenoble University, between January 2005 and January 2007 were retrospectively analyzed. Patients were included in this series if they received a specific antibactericidal treatment.

Results This series included 10 patients (3M/7F; mean age 54.1). The clinical features of ocular inflammation were: bilateral panuveitis, episcleritis, bilateral posterior uveitis, and pars planitis. Tuberculin skin test, chest computerized tomography, BK spurtun, and internal medicine consultation were performed for all patients. The diagnosis of presumed tuberculosis was based upon: history, thoracic imaging, and tuberculin skin test. None had extra-ocular symptoms. Sputum cultures were negative, 2 adenopathy biopsies confirmed the diagnosis. Nine patients received specific antibactericidal treatment, without systemic steroid therapy. All of them improved, no relapse occurred after 1 to 2 years after the end of therapy. In one case, tuberculous specific therapy allowed to taper the systemic steroid therapy.

Conclusion The diagnosis of uveitis associated with tuberculosis is difficult since it depends on a spectrum of indirect signs. Bacteriological identification is rarely obtained. In presumed ocular tuberculosis, antibactericidal therapy may be useful to control intraocular inflammation, with or without steroid therapy.

PCR identification of Rhizobium radiobacter in post-operative endophthalmitis


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Purpose To present 2 cases of PCR identification of Rhizobium radiobacter in post-operative endophthalmitis.

Methods Microbiological identification was carried out using samples from aqueous humor and/ or vitreous. Conventional cultures were performed using a Brain Heart infusion broth. We used broad range subehacterial PCR amplification followed by direct sequencing.

Results In both cases, Rhizobium radiobacter was identified using subehacterial PCR and cultures of vitreous from vitreous tap. An 81-year-old female presented an endophthalmitis 4 weeks after a cataract surgery. Inflammation and infection were controlled after 2 intravitreal antibiotics injections and the final visual acuity was of 20/24 at the one-year follow-up exam. A 75-year-old male who underwent a cataract surgery presented an endophthalmitis 9 days after. This patient was treated by 3 intravitreal antibiotics injections and a vitrectomy. The 6-month follow-up exam showed an optic nerve atrophy with a poor visual outcome (20/120). Both patients had an initial marked anterior chamber inflammation with a hypopyon and a severe retinal vasculitis was observed in the second case.

Conclusion Rhizobium radiobacter is a rare pathogen involved in postoperative endophthalmitis. As it is an environmental soil organism, we may assume that the patient's exposure to outdoor environment and most soil remains the source of this organism. This gram negative rod is resistant to vancomycin and have an intermediate resistance to most antibiotics used to treat post-operative endophthalmitis. PCR allows a specific bacterial identification than do cultures and may help choose the most efficient antibiotics.
# 241

**Is AZOOR an autoimmune disease?**

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**Purpose** Acute zonal occult outer retinopathy (AZOOR) is one of the "white dot syndromes" and is considered a clinically heterogeneous group of inflammatory choroidal retinopathies. The etiology is not yet clear.

**Methods** We present a 50 years female patient with a prior history of migraine. She experienced progressive visual loss and visual field defects in the last 3 years. Preceding each episode she experienced blue flickering photopsias.

**Results** Visual acuity was 0,3 in the right eye and 0,6 in the left eye. Biomicroscopy showed a normal anterior segment, fundus exam revealed pigment epithelial atrophy more pronounced in the worse eye. Electroretinography showed a marked reduction in the photopic ERG and a normal a- and b-wave in the scotopic ERG. MRI demonstrated multiple white matter lesions including a corpus callosum location. Limbal punctuation showed occasional bands. Further tests demonstrated bearing impairment. Therapy was instituted during the three years course of the disease with steroids, immune suppressants and plasmapheresis with visual loss being progressive. New photopsia is currently present.

**Conclusion** The etiology of AZOOR remains unclear. With our patient being one of the very few described in the literature with concomitant multiple sclerosis, the question remains on whether there is an underlying common process of inflammatory autoimmune reactions. Whether treatment is possible, remains to be evaluated.

# 243

**Recurrent giant chalazia in hyperimmunoglobulinemia E (Job's) syndrome**

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**Purpose** To report a case of recurrent, multiple giant chalazia in a patient with hyperimmunoglobulinemia E (Job's) syndrome, a rare autosomal dominant disorder characterized by markedly increased immunoglobulin E levels and recurrent pulmonary and skin infections.

**Methods** A 50-year-old man was referred with a 4-year history of recurrent, multiple giant chalazia in all eyelids. Medications and surgical intervention produced only transient improvement. The patient also had a history of pulmonary and scalp infection.

**Results** Laboratory tests disclosed elevated plasma immunoglobulin E (> 500 IU/ml) and eosinophilia. As a result, based on clinical and laboratory findings, a diagnosis of hyperimmunoglobulinemia E (Job's) syndrome was made.

**Conclusion** Even though rare, recurrent multiple giant chalazia may occur as an ophthalmic feature of the Job's syndrome. The hyperimmunoglobulinemia E syndrome should be suspected in any case of recurrent giant chalazia. Measurement of plasma immunoglobulin E and eosinophils, along with internal evaluation, are essential to establish a proper diagnosis.

# 242

**Comamonas: a not so common agent in hydrogel contact lens wearers**

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**Purpose** To present an unusual agent responsible for a case of keratitis in a hydrogel contact lens wearer.

**Methods** A 16 year old patient, hydrogel contact lenses wearer, presented with red eye. The patient reported red eye with blurred vision and pain for 1 day. In the central cornea, two stromal lesions were found surrounded by an infiltrate. The contact lenses, the lenses box and lenses cleansing products were sent to analyse and Comamonas acidovorans was found.

**Results** The agent isolated was Comamonas acidovorans, a species of Pseudomonas. The patient was treated with local ciprofloxacin, tobramycin and hexamidine dimethane, 0.1%, with remission of the case.

**Conclusion** Cultures and sensitivity studies should be performed in contact lenses wearers. Comamonas acidovorans should be considered as a possible keratitis agent.

# 244 / 2126

**Clinical management of congenital achromatopsia**

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**Purpose** Describe the long term follow-up of children affected by congenital achromatopsia (CA). CA is a rare genetic inherited vision disorder (1:50000) and includes two clinical categories: complete CA and incomplete CA.

**Methods** Fourteen children (9M, 5F) were followed-up and a rehabilitation individual program was carried out. The assessment included: far and near visual acuity (Teller, Lea Symbols, Snellen Charts), refraction, color vision (Farnsworth, Ishihara, Montessori), contrast sensitivity, visual field, slit lamp examination, fundus ophthalmoscopy, electrophysiologic tests (photopic, scotopic and flicker ERG and VEPs) and genetic examination.

**Results** Early onset of nystagmus, low vision and photophobia are first clinical manifestations of CA. The differential diagnosis includes Leber Amaurosis, and congenital nystagmus and albinism. Visual acuity in our patients is around 1/10 and it is worse for near. Refractive errors showed a distribution toward hyperopia (50%) and emmetropia (35%). Macular hypoplasia is present in all cases and electrophysiology showed a reduced or absent photopic ERG, while scotopic system appears normal.

**Conclusion** Due to abnormal or totally absent cone function, which lead to macular hypoplasia, CA shows reduced visual acuity, absent/severely impaired color vision, nystagmus and photophobia. Low vision aids such as telescopic systems and magnification devices along with OCR/ICR software were prescribed after a carefully evaluation. Spectral filters were early prescribed to reduce photophobia and improve vision. The molecular analysis may confirm the diagnosis and give genetic counseling to the family. An early detection of CA is underlined by the importance to carry out a complete and precocious rehabilitative approach.
Family with Brittle Cornea Syndrome

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Purpose: We present a case of a 29-year-old woman presenting with corneal rupture after minor trauma. This is the third person in the same family with corneal rupture and other features of brittle cornea syndrome (BCS). This is the largest known family with this syndrome and the age at corneal rupture is the oldest described.

Methods: A 29-year-old woman presented with left corneal rupture evoked by minor trauma and received left corneal excision and implant. Two of her younger sisters had also presented with corneal rupture when they were 3 and 4 years old. They had also blue sclera and several bone problems and they suffered ear problems. Another sister is known to have bilateral keratoconus, but no history of corneal rupture. All the injuries affecting the family members were provoked by minor trauma.

Results: This is an interesting family with BCS. BCS presents with brittle cornea, blue sclera and red hair. Recurrent spontaneous corneal perforations, sometimes bilateral, are a feature. Other characteristics that have been described are hypermobility of the small joints, moderate skin hyperextensibility without excessive skin fragility, and hearing impairment.

Conclusion: The major differential diagnosis of BCS is Ehlers-Danlos syndrome type VI, since VI Ehlers-Danlos is associated with poorer prognosis. It also needs differentiation from osteogenesis imperfecta. The exact genetic abnormality in BCS is yet to be discovered. A recent article has implicated chromosome 16 and offered some candidate genes, among them the PCOLIN 3 which has been implicated with collagen formation. BCS may be important in medicolegal cases as potential misdiagnosis of non-accidental injury may occur.

Proof of new collagen synthesis in lid skin after Er:YAG laser skin resurfacing with in-situ hybridization

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Purpose: The aim of the study is to prove new collagen synthesis in lid skin fibroblasts and correlate it to laser energy fluence after Er:YAG laser skin resurfacing, using the method of in situ hybridization.

Methods: Lid skin of 9 patients with blepharochalasis was treated with laser at day 7 and day 21 before elective blepharoplasty, when it was excised for further investigation. We used a 2940 nm Er:YAG laser (Fidelis by Fotona) with smooth mode parameters: laser fluence 0.75 – 2.00 J/cm2, spot size 5 mm, repetition rate 20 Hz, pulse length 550 μs, 6 pulses per packet, packet length 250 ms, no overlapping. We designed 2 oligonucleotide RNA probes for pro-alpha 1 and pro-alpha 2 collagen and labeled them with radioactive Sulphur 35. At time of surgery the excised tissue was frozen in dry ice. Tissue sections were incubated with the probes and marked with film emulsion for 10 days. We counted silver grains over specific cells, which were identified with immunohistochemistry.

Results: We noticed significant elevation of pro-alpha-collagen mRNA expression in the laser treated areas of lid skin compared to untreated areas. Pro-alpha-collagen mRNA expression was particularly dense in cells identified as fibroblasts by immunohistochemistry. Pro-alpha-collagen mRNA expression in these cells correlated with laser fluence used in treatment of lid skin.

Conclusion: The method of in situ hybridization brings a definitive proof of new collagen synthesis in skin fibroblasts after Er:YAG skin resurfacing, and it is possible to measure the rate of collagen synthesis as a function of the applied laser fluence.

Correction of congenital ptosis using Prone-up

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Purpose: To determine the functional and cosmetic results of Prone-up ptosis sling material for the correction of congenital ptosis.

Methods: In this prospective study, 13 frontalis sling suspension surgeries were performed by the same surgeon in 12 consecutive children with congenital ptosis. The sling material was sutured to the tarsus through an eyelid crease incision. The eyelid contour, complications during and following surgery were recorded.

Results: The ages of the cases ranged from 1 to 21 years (mean 7.5 ± 6.9 years). The patients were followed up for at least 6 months (mean 16.7 ± 8.4 months). All ptotic eyelids were corrected with acceptable final lid position. Exposure keratitis, wound infection, eyelid contour abnormality or overcorrection was not observed in any patient. One case had trauma to the peribulbar area one month following surgery which resulted in recurrent ptosis and required re-operation. Another patient with recurrent ptosis at presentation had persistent eyelash ptosis following surgery which required an additional surgery. Good to excellent final postoperative height was achieved in 8 patients (9 eyelids), and 2 cases with mental retardation could achieve good height with conscious active brow elevation.

Conclusion: Prone-up sling material is a biocompatible, biointegrable, inset material, which results in cosmetically and functionally satisfactory results following surgery of congenital ptosis. Following surgery, patients learn to use their frontalis muscle to achieve satisfactory eyelid height. This can also be achieved in patients with mental retardation if taught to exert conscious effort to activate the frontal muscle.

Dacryoendoscopy: feasibility and value as diagnostic tool

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Purpose: To access the feasibility and value of endoscopy of the lacrimal duct as a diagnostic method in setosuses of the lacrimal drainage system.

Methods: In this prospective study 50 patients with prolonged complaints of epiphora or history of recurrent dacrocystitis were included. All patients underwent clinical examinations including syringing, digital dacrocystography and dacryoendoscopy before a decision for appropriate surgical treatment was made. Findings of clinical examinations, dacrocystography and dacryoendoscopy were compared in regard of feasibility, localization of the obstruction, particular informations provided and correlation between the three methods.

Results: Dacryoendoscopy could be performed in all patients included in the study. Concerning the localization of stenoses, 48% of the patients were found to have complete post-saccal stenosis, 20% stenoses of the canaliculus, 10% combined complete post-saccal stenosis and partial stenosis of the canaliculus and 2% a partial stenosis of the distal lacrimal duct. In 18% of the patients no definitive location could be found using dacryoendoscopy. The localization of the stenosis determined with dacryoendoscopy correlated in 66.7% with the clinical diagnosis according to syringing, and in 70% with the localization of the stenosis as determined by dacrocystography. Endoscopy could provide additional information about pathologies of the lacrimal system, like lirhitis or polyposis.

Conclusion: Dacryoendoscopy can provide valuable information under direct visualization of the lacrimal system. However it must be doubted whether the benefits of this method may justify to substitute the other well established methods in the diagnostics of dacryostenoses.
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Periocular Er:YAG laser skin resurfacing in ablative, dual mode and smooth mode – a histological comparison

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**Purpose** The aim of the study is to histologically compare Er:YAG laser skin resurfacing using ablative, dual mode and smooth mode parameters.

**Methods** 30 patients with blepharochalasis were treated with a 2940 nm Er:YAG laser (Fidelis by Fotona) at day 0, 7 and 21 before elective blepharoplasty. At time of surgery the irradiated lid tissue has been removed and prepared for histological analysis. The laser settings used in the ablative mode were: fluence 2.5–4.5 J/cm², spot size 5 mm, repetition rate 5–8 Hz, pulse length 300 µs; dual mode: first pass as in ablative, then second pass with fluence 0.5–1.5 J/cm², spot size 3 mm, repetition rate 12–15 Hz, pulse length 300 µs and 60% overlapping; smooth mode: fluence 0.75–2.0 J/cm², spot size 5 mm, repetition rate 20 Hz, pulse length 500 µs, no overlapping.

**Results** We observed deep collagen denaturation at fluences above 1.25 J/cm² and total epidermal damage at fluences above 1.75 J/cm²; complete regeneration of epidermis at day 7 and collagen remodeling up to 240 µm deep at day 21 after smooth mode laser treatment. These changes were statistically significantly different compared to changes after ablative and dual-mode treatment.

**Conclusion** Smooth mode Er:YAG laser skin resurfacing is a safe and effective method producing new collagen synthesis and remodeling, with less epidermal damage compared to ablative and dual-mode resurfacing.

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Frequency of headache and associated eye conditions in Congolese patients

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**Purpose** To determine frequency of headache and associated eye conditions in patients from D R Congo.

**Methods** A case-control study was performed between April and September 2004 and included patients seen at an ophthalmologic clinic in the city of Kinshasa. Cases were all consecutive new patients with headache detected during the study period. Controls were all other patients seen in the same ophthalmologic clinic. Data were collected from 476 patients by medical examination and interview. Ocular examination included measurement of visual acuity, refraction, slit lamp examination and fundoscopy. Other examinations were performed when needed.

**Results** There were 102 patients with headache (cases) and 374 patients without headache (controls), giving a frequency of patients with headache of 22%. Mean age was 39 years (14 for cases and 41 years (19 for controls (P=0.32). There were more female compared to male in cases (71%) than controls (55%) (P=0.001). Eye conditions were found in 91% (89% cases and 35% (95% controls (P=0.018). Cases were more likely to have asthenopia than controls (15% vs 6%, P=0.006). There was no differences between cases and controls in the distribution of ametropia (20% vs 16%, P=0.39), presbyopia (30% vs 21%, P=0.07), pterygium (5% vs 4%, P=0.69). There were no significant differences between cases and controls in the distribution of hypertension, sinusitis and diabetes. The commonest locations of headache in cases were diffuse, the frontal, temporal areas.

**Conclusion** Ocular examination could be necessary in patients with headaches.

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A model of paediatric eye screening program for educators

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**Purpose** Presentation of a paediatric eye screening program which is based on the educators’ performance.

**Methods** 4000 children (n=1-2200 3-16 y.o., n=2200 4-6 y.o.), 10 ophthalmologists and 20 teachers participated in this study. Children were tested for visual acuity (VA) using the NK test and manifest horizontal strabismus using the Hirschberg test. Criteria for further evaluations were: VA<0.55 and/or manifest strabismus.

**Results** In n=2200, doctors found 105 children (4.77%) appeared with VA<0.55, 198 children (81.4% with VA≥0.55 and 157 children (7.13%) with VA≥0.55. In n=1 teachers found 191 children (84.6%) appeared with VA<0.55, 179 children (81.78%) with VA<0.55 and 210 children (93.4%) with VA≥0.55. In n=1, doctors found 211 children (95.95%) were orthophoric, 62 children (28.1%) were esotropia and 27 children (12.3%) were exotropia. Teachers found 205 children (92.22%) were orthophoric, 106 children (48.1%) were esotropia and 43 children (1.97%) were esotropia. In n=2200, doctors found 164 children (7.4%) appeared with VA<0.55, 169 children (77.05%) with VA<0.55 and 341 children (15.7%) with VA>0.55. In n=2, teachers found 160 children (7.27%) with VA<0.55 and 168 children (74.5%) with VA>0.55 and 401 children (18.22%) with VA<0.55. In the same sample (n=2), doctors found 212 children (96.4%) were orthophoric, 62 children (28.1%) were esotropia and 17 children (0.79%) were exotropia. Teachers found 204 children (92.77%) were orthophoric, 46 children (2.08%) were esotropia and 95 children (4.32%) were exotropia.

**Conclusion** In a screening eye program false positive referrals are more welcome than false negative especially in a very cost effective program as this one is. We recommend this model in countries where no paramedics as screeners exist.
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Retinal vein occlusion signals a significant risk of mortality in the Beijing eye study


Purpose The purpose of the present population based investigation to assess whether retinal vein occlusion (RVO) may be associated with an increased mortality in the years following the event.

Methods The present investigation consisted of 8669 eyes (97.0%) of 4335 (97.7%) subjects for whom readable fundus photographs were available. For our study recent retinal vein occlusion (RVO) was characterized by the presence of retinal edema, optic disc hyperemia or edema, scattered superficial or deep hemorrhages, and venous dilation. Old retinal vein occlusions were characterized by occluded and sheathed retinal veins.

Results Out of the 4335 subjects, 3194 (73.7%) subjects returned for follow-up examination while 134 (3.1%) subjects were dead and 1005 (23.2%) subjects did not agree to be re-examined or had moved away. In binary logistic regression analysis, mortality was significantly associated with age (P=0.0019, 95%CI:1.001,1.12) and RVO (P<0.007, 95%CI:1.01,6.27) including subjects with an age >70 years or <65 years, respectively, the association between RVO and mortality became further more significant (P=0.007, 95%CI:1.46,10.54) and P<0.0001,95%CI:1.10,24.95, respectively).

Conclusion The results suggest that retinal vein occlusion, particularly in relatively young persons, may signal a significant risk of mortality. It confirms a recent combined evaluation of the Blue Mountain Study and the Beaver Dam Study in which, as in the present study on Chinese, patients with a retinal vein occlusion, particularly if the persons were younger than 69 years, had a significantly increased mortality.

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Phenotype characteristics of patients with USHER syndrome type 2

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Purpose To investigate whether USH2A and USH2C genes are clinically distinguishable based on data described in the literature.

Methods In a patient group diagnosed clinically as USHER syndrome type 2 (35 patients, aged 6-64 yrs) best corrected visual acuity (BCVA), static visual fields (Octopus 101), full field and multifocal electroretinograms (RETIport and RETIscan, Roland Consult) and the structure of the central retina (optical coherence tomography, using Stratus OCT) were examined.

Results BCVA: LogMAR 0.3 or better in 19, 0.3-1.0 in 13 and worse in 3 patients. Radius of the residual central field was between 25’ and 10’ in 11, between 10’ and 5’ in 21 patients and less than 5’ in 3 patients. Full field ERG both rod and cone responses were extinguished in 29 patients, while residual 30 Hz flicker cone activity was detectable in 6 patients. MERRF: subnormal b-wave amplitudes were measured in the central 3 rings in 11 patients, 2 rings in 16 patients, in the central ring only in 3 patients and in 5 patients no response was recordable. OCT was carried out in 18 patients; decrease in TNV in 10 patients; blunting of the foveal pit in 3 and cystic intraretinal changes in 2 patients were seen.

Conclusion This retrospective examination does not allow a correct differentiation of the two USher subtypes. In the majority of patients clinical symptoms indicate the phenotype of USH2A. Three patients (with the most severe clinical condition) might represent a different genetic subtype.

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Identification of a novel VMD2 mutation in Slovene family with best vitelliform dystrophy

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Purpose To report a clinical phenotype and a novel VMD2 gene mutation in a Slovene family with Best vitelliform dystrophy.

Methods Father (51 yrs, VA: 0.1, wo) and his two sons (31 yrs, VA: 1.0 RE, 1.0 LE; and 17 yrs, VA: 0.6 RE, 0.2 LE) with abnormal EOG and clinical features of Best dystrophy were tested with static perimetry, pattern ERG and multifocal electroretinography. Autofluorescence imaging of the fundus was done by HRA. Screening for mutation was done by the non-isotopic high resolution single stranded conformation analysis (SSCA) after amplification of each exon by the polymerase chain reaction (PCR). Primers for VMD2 were described by Petrikulin.

Results A new mutation was found with G to C substitution at position 147 in VMD2 gene, altering nonpolar glycerine to a bigger and positively charged arginine at codon 15. The change altered electric balance of the mutated protein. Four other changes in these family were observed as well (P416P, T470I, T536T, S519S), but have already been described by other groups and presumably did not alter the predicted amino acid sequence of bestrophin protein. AF imaging showed different patterns in each patient according to the stage of disease - central hyperfusional lesions in early stages and central hypofusional regions being surrounded with hyperfusional rings in later stages of the disease. Electrophysiology testing showed reduction of pattern S50 and N55 amplitudes in those with lower VA. Reduction of P1 amplitude of mERG was mostly observed in the inner three concentric rings already in the early stages of disease.

Conclusion A novel disease-causing mutation (G15R) was found in the exon 2 in VMD2 gene in a Slovene family with BVD.

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Gene expression changes associated with endothelial cell loss during corneal organ culture

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Purpose Prior to transplantation corneas are stored in organ culture for up 4 weeks. However, up to 30% of corneas are discarded, mainly due to a poor endothelial cell count. In this study we have investigated the genes expressed during the process using micro array technology.

Methods Human corneas were stored in organ culture and the endothelial cell numbers where assessed after 10 days. RNA was extracted from 3 corneas with a low and 3 with a high cell count. The RNA samples were converted to cDNA using a Poly A PCR amplification protocol. These were used to probe Affymetrix whole genome micro array chips.

Results Results showed over 1000 genes are either up or down regulated by the endothelial cells of corneas with a low cell count compared to corneas with high counts. As expected there are genes involved in apoptosis, cell signalling, cell cycle, extracellular matrix, cell adhesion and cytoskeleton genes. However, of more interest are the genes involved in water channels and transport. For example we have identified several aquaporin genes (AQP) which are integral membrane proteins that serve as channels in the transfer of water across the membrane. AQP1 is increased and AQP5 is decreased in the corneas with low cell counts. It is likely that the changes in expression of these genes further increase the swelling of the corneas in culture and cause damage to the endothelium leading to higher levels of cell death in the affected corneas.

Conclusion These results add to our understanding of the effects of cell culture conditions on the corneal endothelium. A better understanding of endothelial cell loss in culture would lead to improved culture protocols and increase the number of corneas available for transplantation.
* 257 Development of lentiviral vectors for gene therapy for Usher syndrome type 1B

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Purpose Usher 1B, one of the major subtypes of a combined blindness and deafness disease, is caused by mutations in the MYO7A gene, which encodes a large unconventional myosin expressed in the retinal pigment epithelium (RPE) and photoreceptor (PR) cells. This study aims at developing viral vectors expressing the wild type human MYO7A at an adequate level in order to rescue cellular phenotypes of MYO7A mutation.

Methods The full-length (7 kb) human MYO7A cDNA was cloned into the third generation, self-inactivating lentivector under different promoters and enhancers. Human genomic 4 kb DNA fragment including exon 1 through 2 was cloned by PCR. Activities of different promoters and enhancers were tested by reporter assays using ARPE-19 cells. Previously identified Myo7a-null phenotypes in shaker-1 mouse were used to test the efficacy of various lentiviruses.

Results Lentiviral vectors could successfully transduce large genes (up to 7.6 kb) in vitro and in vivo for the purpose of gene therapy. Reporter assay indicated that regions with a suppressor activity and an enhancer activity existed within intron 1. The CMV promoter drove excessive MYO7A expression in the RPE, and thus caused cellular death. A chimeric promoter that consists of partial CMV promoter with 160 bp MYO7A enhancer could direct moderate levels of gene expression in RPE and PR, in vivo, and rescued a number of phenotypes in the mutant mice.

Conclusion These results illustrate the importance of regulating transgene expression levels in achieving therapeutic outcomes. They demonstrate the efficacy of lentivirus-mediated expression of the large human MYO7A cDNA as a gene therapy strategy for correcting the MYO7A deficiency underlying Usher 1B.

* 258 Mapping candidate genes for familial high myopia in a polish population

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Purpose Myopia is the most common of all ocular conditions. The etiology of myopia is not known in high myopia (myopia in excess of -6.0 diopters), genetic factors appear to play a predominant role. The purpose of this study is to map a gene(s) responsible for high myopia in Polish families.

Methods To date, we have collected blood and purified genomic DNA from 418 individuals from 51 Polish unrelated high myopia families. A simulation study was performed to evaluate whether there is sufficient power to detect linkage in the ascertained pedigrees. Then, using DNA samples from 22 families we performed two series of experiments. First, to exclude specific syndromes associated with myopia in Polish families, we tested linkage to Stickler syndrome, Marfan syndrome, Knobloch syndrome and juvenile open angle glaucoma. Next, we searched for linkage to the high myopia-associated loci [18p11.31 (MYPP2), 12q21.23 (MYP3), 7q36 (MYP4), 17q21.23 (MYP5), 3q17, 4q22-24 (MYP71), 3q26 (MYP8), 4q21 (MYP9), 8q23 (MYP10) and 10q21.1]. Additionally we performed the genotyping to test the linkage for mild-moderate myopia established in Ashkenazi Jewish families [22q12 (MYPP6) and the PAOX gene region reported for dizygotic twins [11p13 (MYPP7)].

Results Simulation studies show that sufficient power exists to establish linkage in collected pedigrees (n=0.4; EMLD-412). Linkage to Stickler syndrome, Marfan syndrome, Knobloch syndrome, juvenile glaucoma loci and to any of the candidate genes were excluded. Genome wide screen is in progress.

Conclusion We have identified, collected and characterized a large cohort of polish families with high myopia and excluded previously suggested principal genetic cause of this phenotype.

* 260 / 2246 Fixation stability during the Rarebit Fovea Test

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Purpose To evaluate the fixation stability in normal subjects during the Rarebit Fovea Test (RFT). The RFT is a computerized test designed to evaluate the integrity of the central retinal detector matrix.

Methods 11 healthy subjects with visual acuity ≥ 1.0, were examined, using the standard RFT setup, i.e. stimulus presentation on a 17 inch computer screen at a viewing distance of 2 m. The test principle is to briefly present one or two very small (< 0.5') and bright dots in the central 4° visual field. The subject responds by clicking the computer mouse once or twice. The test time is approximately 90 sec and audio-stimulation is given each time the fixation target is rotated in order to enhance central fixation. Fixation stability was measured during the RFT test using the PowerRaterfactor® (Purkinje image eye-tracking). One eye in each subject was tested. All measurement data were analyzed using SPSS and Matlab.

Results All subjects had normal RFT test results, i.e. close to 100% of the presented dots were perceived. 97% of the testing time fixation was maintained within 1.38° and 100% of the time within 2.56°.

Conclusion Normal subjects appear to maintain stable fixation during the RFT. Further studies of patients with minor macular changes are underway.
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**Methodology of retinal straylight assessment**

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**Purpose**
The psychophysical technique of ‘Compensation Comparison’, embedded in the C-Quant instrument for clinical assessment of retinal straylight will be demonstrated. Retinal straylight is the cause of complaints such as glare hindrance and contrast loss. It results from disturbances in the optical media. Its assessment helps to decide on surgery of (early) cataract, or to evaluate corneal or vitreal problems.

**Methods**
The subject’s task is to compare in 20 short presentations two flickering half fields, and to indicate with push buttons which one flickers more strongly. Added value in comparison to visual acuity was assessed in 2422 active drivers of the European GLARE study. Repeatability of the obtained straylight value was tested for 17 naive subjects and several groups of patients. Absolute measurement precision of the C-Quant was tested using a scattering sample with known straylight value in front of the eyes of the 17 naive subjects.

**Results**
Many individuals were found to suffer from increased straylight that is undetected in visual acuity. In 6% of cases with decxal visual acuity > 0.5 straylight was increased more than 4 times compared to normal. A psychometric function was derived and fitted to the patient responses, resulting in a straylight value including a reliability estimate. Repeated measures standard deviation for the method is about 0.07 log units, to be compared with differences of 1.0 log units or more units with (early) cataract or corneal disturbances. The check using known scatter samples showed virtually perfect (0.01 log units difference) correspondence.

**Conclusion**
Absolute precision and repeated measures standard deviation is very good compared with differences in the population. Straylight measurement promises important extra diagnostic power.

**Commercial interest**

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**Which color vision test should be used in progressive cone dystrophy?**

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**Purpose**
The early manifestation of progressive cone dystrophy (COD) can remain unrecognized due to the relatively normal macular appearance. Color vision testing can be very useful as a first diagnostic step. The many available color vision tests have different benefits and shortcomings. We aimed to identify which test would be preferred to use in a clinical setting as a first step towards diagnosis of COD.

**Methods**
We compared patients (n=18) derived from the ophthalmo-geneic unit of Erasmus Medical Center and University Medical Center Nijmegen, with various levels of cone dysfunction. Golden Standard for diagnosis of COD was a diminished photopic ERG and a relative central scotoma on Goldmann perimetry. Controls (n=13) were patients from these clinics with other diagnoses or healthy companions of COD patients.

We estimated sensitivity and specificity of the Ishihara test, Lanthony Desaturated and Saturated Panel D15 test, the Hardy-Rand-Rittler (HRR) pseudo-isochromatic plates, and the Nagel anomaloscope. We analyzed sensitivity, specificity and the predictive value with receiver operating characteristic curve (ROC).

**Results**
The HRR test had the highest sensitivity and specificity for protan and deutan axes. HRR and Ishihara had the highest predictive value. Lanthony Panel D15 test did not have an additional predictive value for severe color vision defects. The Nagel anomalouscope was not reliable due to low specificity. Its results showed high variations among both healthy and afflicted individuals.

**Conclusion**
The HRR test was the most useful for COD. This test had the highest sensitivity in detecting early dysfunction of all three cone types, and it adequately quantifies the level of cone dysfunction in the course of the disease.
# 265

**Rarebit Fovea Test and OCT findings in diabetes**

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**Purpose** To evaluate foveal structure and function using optical coherence tomography (OCT) and the Rarebit Fovea Test (RFT) in diabetic patients without known maculopathy or retinopathy.

**Methods** Forty-two patients with diabetes mellitus (DM) were selected from the screening records at St Erik Eye Hospital. Inclusion criteria were no previously known macular or other retinal changes and best corrected visual acuity ≥ 1.0. These patients and 42 healthy controls were examined with the recently developed RFT and retinal thickness was measured using OCT. Lens thickness and light scatter was evaluated by Scheimpflug photography.

**Results** Significantly more DM subjects (12/42) had a subnormal RFT result compared to the controls (2/42) (p = 0.007). The retina in the inner OCT zone was significantly (p=0.05) thinner in DM patients with subnormal RFT compared with the controls. No difference between groups were observed in lens thickness or light scatter.

**Conclusion** Macular changes detected by conventional screening methods could be demonstrated using both structural and functional tests in a subgroup of patients with DM. Prospective studies are underway to evaluate the prognostic implications.

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**Foveal function in children treated for amblyopia**

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**Purpose** To evaluate foveal function in children treated for amblyopia using best-corrected visual acuity (BCVA) and the Rarebit Fovea Test (RFT).

**Methods** Nineteen children, aged 7-9 years, previously treated for monocular amblyopia were examined with BCVA and RFT. Twenty-three healthy, age- and gender-matched children served as controls. BCVA was measured with a line letter chart. The foveal function was measured with the computerized RFT, designed to detect minor defects in the retino-cortical detector matrix. In all amblyopic eyes, the non-amblyopic eye was tested first.

**Results** The median decimal BCVA was significantly lower in the amblyopic eyes (0.8, range 0.2-1.0) compared to the non-amblyopic eyes (1.0, range 0.8-1.3, p = 0.0006) and to both-eyes in the control group (1.0, range 1.0-1.3, p = 0.0001 and p = 0.0001, respectively). The RFT results were significantly (p=0.0012) lower in the amblyopic eyes (second tested) compared to the second, but not the first, tested eyes in the control group. In this group the second tested eye showed better RFT results compared to the first tested eye, but the difference was not statistically significant. A weak (r2=0.25) but significant (p=0.028) correlation was found between BCVA and RFT results in the amblyopic eyes only.

**Conclusion** The study demonstrated a difference in foveal function, measured with RFT, in amblyopic eyes, when tested after the non-amblyopic eye, compared to the second tested eye in age-matched controls. This finding may be interpreted as a sign of reduced function in the amblyopic eye or as lack of training effect in amblyopic subjects.

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**The influence of pupil size, accommodation and higher order aberrations on the retinal image of a Rarebit Fovea Test-stimulus**

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**Purpose** To study the influence of pupil size, accommodation and higher order aberrations on the retinal image of the small test stimulus (<0.5’’) used in the Rarebit Fovea Test (RFT).

**Methods** Eleven subjects aged between 22-36 years participated. All subjects had a corrected visual acuity of 6/6 or better and a refractive error within ± 6 diopters. A PowerRefractor TM measured pupil size and accommodation continuously in all subjects during performance of the RFT. Aberrations were measured with a Zywave aberrometer and Matlab software was used for analysis.

**Results** Mean variation in accommodation was 0.4D (range 0.22-0.61D) and mean pupil size was 6.1 mm (range 5.0-7.8 mm). The aberrations induced a smearing of the retinal image to -3.5x the stimulus size. When the variation in accommodation was added the total smearing of the retinal image increased to ~4x the stimulus size.

**Conclusion** The minimum retinal image size of a RFT stimulus is given by aberration and defocus rather than the actual size of the object (stimulus).

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**Macular function in severe disbinocular amblyopia**

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**Purpose** To evaluate the mechanisms of the visual impairment in severe disbinocular amblyopia (SDA).

**Methods** 41 children with SDA 5-17 years old, visual acuity 0.03-0.1; paraventral, paramacular, peripheral fixation were examined. Mixed, pattern, macular multifocal ERG examination (MNB Moscow and Roland Consult Germany) and topography, contrast, colour sensitivity and spatial contrast sensitivity were performed.

**Results** The impairment of the color sensitivity to unsaturated red, green and blue was found. The most changes were found for the green in ‘‘high’’ from the macula. No changes in the mixed, macular and ficker 30 Hz ERG was found. Although some patients showed a supernormal a-wave of the macular ERG as well as a subnormal b-wave, supernormal ficker 30 Hz ERG and moderately subnormal pattern ERG. Mi ERG showed the decreased rates of retinal density in the 1st and 2nd ring with normal rates of N1 and P2 in all central hexagons. SDA with non-central fixations in 2D display is manifest with the zone of maximal bioelectric activity noticeably shifted from the fixation point. Together with the stable central fixation there is a near to the normal retinal density in central rings as well as with the eccentricity, while the maximal bioelectric activity at the non-central fixations coincide with the presumed projection.

**Conclusion** Changes in the color and contrast sensitivity and in the retinal bioelectric activity in SDA has a functional character. Rate of the found changes differs from those in other retinal and optic nerve pathologies and retrochiasmal changes in children. There might exist an abnormality of photoreceptor and neuronal interactions among other mechanisms of visual function impairment in ADA.
Correctable and non-correctable refractive error in a population of young men in Poland

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Purpose To evaluate the prevalence of non-correctable refractive error in a representative sample of Polish young men.

Methods A retrospective study of medical records of a Military Medical Commission of Lodz, Poland. Distance visual acuity (VA) was assessed in 105017 men examined during period 1993-2004. Visual acuity was checked on Snellen's charts without vision correction. We chose for the study a representative sample of 994 individuals. Refraction data were obtained of those men who had impaired distance vision presenting VA of below 6/12 in any eye. Non-correctable refractive error was defined as the best corrected visual acuity (BCVA) of below 6/12 in one or both eyes. We use statistic analysis to estimate the results in a whole population of young men examined during researched period.

Results Mean age of researched individuals was 22.46 +/- 4.29. Overall 11.28% subjects had impaired distance vision. After refract, 90.15% of them could achieve VA of 6/12 or better with corrective lenses. Non-correctable refractive error was found in 9.85% of men with impaired distance vision. Extrapolating these results to the whole population we estimate that 1.21% of Polish young men have non-correctable refractive error. BCVA of below 6/12 in one or both eyes. Correctable refractive error was mainly due to myopia (78.10% right eye and 81.02% left eye).

Conclusion The overall non-correctable refractive error had a relatively low prevalence (1.21% of population). Appropriate refractive correction improved distance VA in 90.15% subjects with impaired distance vision. The commonest refractive error was myopia.

Function of macular area in retinopathy of prematurity

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Purpose To assess the bioelectric activity of the retina at different stages of the retinopathy of prematurity (RP).

Methods 21 children with RP (stage 1-4, 6-14 years old, born at 27-32 week of gestation with the birth weight of 730-1800g) were examined. In 4 of children the prophylactic laser coagulation of avascular retina was performed in the active phase. Visual acuity (VA) at the stage 1 of RP amounted to 0.7%, at the stage 2 0.5%; at the stage 3 0.25 and at the stage 4 0.02. Macular (MIN Moscow) and mfERG (Roland Conault Germany) were examined.

Results There was no correlation between VA values and parameters of multifocal (mf) and macular (m) ERG. Patients with RP of stage 1 showed a moderate reduction of b-wave magnitude of mfERG at its normal latency. This correlated with mfERG data in central hexagons 1.5 degrees. The magnitude and latency of mERG were changed to a great extent in RP patients of stages 2-3. The waves N1 and P1 of mERG were also heavily decreased at normal latency. The patients with severe retinal abnormalities, like retinal detachment, have subnormal mfERG values with prolonged latency, and moderate decrease of retinal density in the central ring and considerable changes with eccentricity in mERG.

Conclusion RP patients 1-4 stages showed considerable impairment of macular function independent of the ophthalmoscopic changes. Even occult or weak-manifested of the diseases in the macula might be accompanied with the moderate decrease of macular bioelectric activity including the abrupt abnormalities of the electrogensis and neuronal interactions in the macular area. Decline VA don't always had relation with RP. The pathophysiological rationale of the latter needs to be elucidated in the future studies.

Functional characteristic of the retina in glaucoma

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Purpose: To investigate bioelectric activity of the retina in normal subjects and patients with primary open angle glaucoma (POAG).

Methods: Methods: 40 eyes of 20 normal subjects and 80 eyes of 45 patients with different stages of glaucoma were examined with electroretinogram (full field, flicker, pattern, multifocal ERG, ERG to long duration stimulus and chromatic ERG to red, green and blue stimuli). Intraocular pressure was 21 mm Hg or less in all patients. All patients were also tested on HRT II.

Results: Full field ERG recordings showed a decrease of b-wave amplitude and an increase of implicit time in advanced and late stages of POAG. Macular chromatic ERG to red stimulus of 12’ area was subnormal in all stages of POAG (p<0.01). The density of retinal responses of multifocal ERG was progressively decreased in all stages. An amplitude of P50 component of pattern ERG in initial stage of glaucoma was decreased (p<0.05). In advanced stage of glaucoma a significant decrease of amplitudes of P50 and N95 components and prolonged implicit time of N95 component were observed. The off-component of long duration stimulus flash ERG in all stages was subnormal. The on-component in early stage was supernormal, whereas in advanced and late stages was subnormal. A damage of off-cone system of the retina was the first symptom of retinal changes in glaucoma. In initial stage of glaucoma ERG symptoms of glaucomatous neuropathy were first detected even before changes on HRT test.

Conclusion The retinal pathology presented in ERG symptoms may probably reflect some pathogenic mechanisms of development of glaucomatous damage, including excitotoxic factors and ischemia.
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**Estimation of a safety limit for ultraviolet radiation-B (UVR-B)-induced cataract in an in vivo pigmented guinea pig model**

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**Purpose** To establish a threshold measure, maximum tolerable dose (MTD), for avoidance of UVR-B-induced cataract in the pigmented guinea pig.

**Methods** Thirty female guinea pigs, five to nine weeks of age, were divided into 5 groups of 6 guinea pigs each. Pupils were dilated bilaterally before exposures. The guinea pigs were unilaterally exposed to UVR-B under anesthesia in vivo, receiving between 0 and 849 kJ/m² over a duration of 60 minutes. Each guinea pig was sacrificed 24 hours after exposure. Lenses were extracted and photographed in vitro. Intensity of forward light scattering was measured for each lens. The data were analyzed with regression using a second order polynomial model, considering the first order term to be 0. The applied MTD concept is based on the UVR-B dose response curve obtained for the pigmented guinea pig.

**Results** All of the guinea pigs exposed to UVR-B developed cataracts in the anterior subcapsular region. MTD for avoidance of UVR-B-induced cataract was estimated to be 690 kJ/m² in the pigmented guinea pig.

**Conclusion** The MTD for avoidance of UVR-B-induced cataract in the pigmented guinea pig is significantly higher than the threshold obtained by Pits et al. in the pigmented rabbit. MTD is an appropriate method for estimation of toxicity for UVR-B-induced cataract in the guinea pig. The pigmented guinea pig is less sensitive to UVR-B exposure than the pigmented rabbit, pigmented rat, and pigmented mouse.

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**Age dependence of cataract induced by ultraviolet radiation-B in mice**

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**Purpose** To investigate the CS7BL/6 mouse if there is an age dependence of the dose response function for in vivo UVR-300 nm induced forward light scattering in the lens.

**Methods** Each of four age groups of 25 mice aged 1, 6, 12, or 24 weeks were randomly distributed on five age group specific UVR-B dose levels. The dose levels selected for each age group were derived from the expected maximum tolerable dose (MTD). Expected MTDs were set to 1.9, 3.2, 4.8, and 6.0 kJ/m² for the 3, 12, and 24 weeks mice, respectively, based on published data for the albino Sprague Dawley. Each animal was unilaterally exposed to UVR-B to the pre-determined dose, delivered during 15 minutes. All mice were sacrificed two days after exposure and both lenses were extracted for: microscopic imaging in incident illumination against a grid and in dark-field illumination, and measurement of intensity of forward light scattering. The difference of intensity of forward light scattering between the exposed and the contralateral not exposed lens was fitted against dose received using regression based on a second order polynomial model.

**Results** Two days after exposure, subcapsular opacities were observed in the exposed lenses from all dose groups except at 0 kJ/m². In all age groups, the difference of intensity of forward light scattering increased with increasing UVR-B dose. The increase was age dependent.

**Conclusion** In the pigmented CS7BL/6 mouse, an increasing in vivo dose of UVR-300 nm induces an increasing intensity of forward light scattering that is age dependent in the age interval 1-24 weeks. This finding should be considered in future design of experiments on UVR-effects to the mouse lens.

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**Safety limits estimation for ultraviolet radiation induced cataract in different age groups**

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**Purpose** To investigate the effect of age on ultraviolet radiation induced cataract and establish the safety limits for different ages (rats and mice).

**Methods** A total of 80 female Sprague-Dawley rats were included in four age-groups: 3, 6, 10 and 18 weeks, and of 100 female C3H mice were included in four age-groups: 3, 6, 12 and 24 weeks. Each age group was divided into 5 subgroups with different UVR exposure doses. The animal were unilaterally exposed to ultraviolet radiation [λmax=300 nm, B6t=10 nm]. The incident dose on the cornea was 0 ~ 8 kJ/m². The animal were sacrificed and both lenses were extracted one week after exposure. The intensity of forward light scattering were measured and photographs were taken. The safety limit for ultraviolet radiation induced cataract for different age groups was estimated by the maximum tolerable dose, which based on the dose-response function of different age groups.

**Results** Maximum tolerable dose for 3, 6, 10 and 18 weeks old rats was estimated to 1.4, 2.0, 3.8 and 4.6 kJ/m² respectively, for mice of 3, 6, 12 and 24 weeks was 2.0, 3.2, 4.1 and 5.2 kJ/m² respectively.

**Conclusion** Young animals were more sensitive to UVR than old ones, the age dependent trend hold the same for rats and mice. Age should be considered when estimating the risk for UVR induced cataract.

**304**

**A new universal rat restrainer for in vivo exposure to UVR without anesthesia**

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**Purpose** Immobilization of rats is required in many psychological and physiological experiments with rats. The aim was to invent a universal device allowing for adaptation of rats of wide age range and to maximize convenience for in vivo exposure to ultraviolet radiation under un-anesthetized conditions.

**Methods** Eighty six-week-old Sprague-Dawley albino female rats were progressively familiarized daily with the restraining device seven days prior to exposure in order to acquire conditioned reflex of place and reduce stress of the awake rodents. In this training period, the animals were handled in the rat restrainer for 5 to 15 minutes during the first day and restrained for 5, 10 and 15 minutes during the second, third and fourth days, respectively. After initial habituation ten minutes preceding the exposure, the animal was fixed in the rat restrainer. Each animal was unilaterally exposed to 8 kJ/m² UVR-300nm for 15 minutes without narcosis. After exposure the animal was unfixed and then replaced into the cage.

**Results** All rats were acclimatized to the limited activities in the rat restrainer during exposure time. The device prevents both intensive head movement and body rotation, making the device effective for in vivo exposure to UVR without anesthesia.

**Conclusion** The currently designed rat restrainer reduces mobility of rats without need of sedatives, enabling our device to be useful in future projects involving chronic repeated in vivo exposure of the eye to ultraviolet radiation.
# 305
**Evolution of light scattering after in vivo close to threshold dose of ultraviolet radiation 300 nm**

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**Purpose** To determine the evolution of light scattering in the albino rat lens after in vivo close to threshold UVR-300 nm.

**Methods** Altogether 4 groups of 20 6 weeks old albino Sprague-Dawley rats were exposed unilaterally in vivo to 8 kJ/m² UVR-300 nm. The animals were sacrificed at 1, 7, 48 and 336 hrs after exposure. Exposure to UVR, depending on group belonging and the lenses were removed for macroscopic imaging of dark-field anatomy, and quantitative measurement of intensity of forward light scattering.

**Results** The intensity of light scattering increased exponentially declining with a rate constant (1/k) of 71 hrs and an asymptote maximum light scattering of 0.16 °ECD. This is consistent with findings for a dose of 30 kJ/m² although the current time constant was higher. There was an indication of a transient increase of light scattering on the contralateral side peaking at 7 hrs after exposure. There was an indication for some repair at 336 hrs.

**Conclusion** Light scattering evolves quicker, the higher the dose, but increases exponentially declining towards an asymptote. The current study suggest that observations of damage from close to threshold in vivo UVR exposure should be made at 1 week after exposure in order to detect the maximum damage.

# 306
**Resolution visual fields in children surgically treated for congenital cataract**

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**Purpose** To evaluate best-corrected visual acuity (BCVA) and high-pass resolution perimetry (HPRP) visual fields in children surgically treated for congenital cataract.

**Methods** BCVA and HRP were recorded from 10 aphakic children, aged 10-15 years and surgically treated for bilateral dense cataract before the age of nine months, and from six aphakic children, aged 10-15 years and surgically treated for bilateral partial cataract at ages between 6 months and 9 years. Twenty-three healthy children, 11 years of age, served as controls.

**Results** The aphakic children had significantly (p < 0.0001) lower BCVA in their best eye (range 0.1-1.3) compared to controls (range 1.0-1.6). Two aphakic children were visually disabled according to WHO. The children with previous dense bilateral cataract showed significantly (p < 0.05) lower sensitivity in their resolution visual fields (from their best eyes) compared to both controls and children with previous partial cataract. The dense cataract group also showed significantly (p < 0.05) smaller difference between the innermost (5 deg) and the outermost (20-30 deg) eccentricity locations in their visual fields than the other two groups.

**Conclusion** Dense cataract, even when surgically treated before the age of nine months, causes persistent impairment of spatial vision both in the fovea and the visual field. Partial cataract appears only to reduce visual acuity.

# 307
**Modeling fluid mixing within the crystalline lens during accommodation**

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**Purpose** Historically, cataract and presbyopia are treated as separate entities. However, it has recently been hypothesized that as the ageing lens loses its ability to change shape, this reduces the mixing and transport of nutrients and antioxidants within the lens that normally occurs as the young, flexible lens alters its shape during accommodation. Such a loss of antioxidant mixing may render the lens more susceptible to cataract formation.

**Methods** We have developed a 3D cubic Hermite finite element model of the human lens to investigate the effect of increasing lens stiffness on fluid movement. Data obtained from previous studies are used to construct an anatomically accurate model. A spherical polar coordinate system is used to provide an accurate physical representation and as a framework to examine mechanics. Polynomial functions are used to define the lens surfaces. A lens capsule thickness and material properties of lens tissue are taken from the literature. Tension is applied via zonules to simulate accommodation.

**Results** Preliminary results verify that the change in shape of the lens surfaces (and the change in optical power) associated with displacement of the lens equator are similar to results from other published models and fall within the physiological range (i.e. using parameters for 30 yo. lens, approx 25 diopters per mm change in equatorial radius). We are currently investigating the fluid movements associated with these changes.

**Conclusion** Our lens model provides a basis for investigating fluid mixing in the young lens during simulated accommodation and also the reduction of fluid movement in stiffer, older lenses.

# 308
**Ultrastructure features of lens epithelium in intumescent white cataract**

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**Purpose** To analyze the ultrastructure of lens epithelium in intumescent white compared to nuclear cataract and to compare the morphological features of the lens epithelium in intumescent white cataract to the mechanism of cortical cataract formation.

**Methods** Samples of anterior lens capsule of 27 eyes with intumescent white and 27 eyes with nuclear cataracts were analyzed for ultrastructure of the lens epithelium by transmission electron microscopy. The Pearson Chi-square statistics was done on the collected data.

**Results** The lens epithelium of both groups showed similar changes: multi layer epithelium, detached epithelium, free spaces between the cells, intracellular vacuoles, degenerated cells, dark cells and star-shaped nuclei. Three samples of intumescent white cataract had extensive degenerative changes with unrecognizable lens epithelium and were not included in the statistical analysis. Statistic analysis of the ultrastructure findings showed that the degenerated epithelial cells were more frequently present in the intumescent white than in nuclear cataract. There was also a negative correlation between the free spaces between the cells and intumescent white cataract.

**Conclusion** The study proved that degenerative changes of lens epithelium in the intumescent white cataract are more frequent than in the nuclear cataract. This could be in concordance with the pathophysiological process of the cortical cataract formation, where dysfuction of the ionic pump on the cell membrane results in osmotic disbalance, cell swelling and destruction. The insignificant findings: multi layer epithelium and detached epithelium; and even the negatively correlated finding: free spaces between the cells could all be artifacts. Further analysis has to be done to prove this.
### 309

**Modulation transfer function in refractive/diffraction multifoal intraocular lenses in vitro**

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**Purpose** The optical quality of two types of multifocal, hybrid refractive-diffractive, intraocular lenses (IOL) is analyzed in vitro. Following the EN ISO international standard, the IOLs’ modulation transfer functions (MTF) are determined for both distance and near vision. The behaviour of these curves when the pupil size varies is studied.

**Methods** The IOLs analyzed were: AcrySof ReSTOR SN60BD (Alcon) and Tecnis ZM900 (AMO). The MTF was calculated from the cross-line spread function recorded with the OPAL Vector System (Image Science Ltd. Oxford) by using fast Fourier transform techniques. The artificial eye model uses simulates in vivo conditions of the anterior chamber including an artificial cornea and a physiological solution where the IOL were positioned. Following pupil sizes were analyzed: 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 mm.

**Results** The MTFs of the two multifocal IOLs analyzed show a good image quality for both in all conditions analyzed. However, a monofocal IOL with the same power (22.5 diopters) provides a better image quality for distance vision. The Strehl ratio of the two multifocal IOLs decreases when pupil size increases, for both distance and near vision. To the contrary, the average modulation (from 0 to 100 c/mm) of the two multifocal IOLs remains approximately constant for all the pupil sizes.

**Conclusion** The image quality provided by the two multifocal refractive-diffraction IOLs is very similar, although the quality with ReSTOR IOL is slightly better than with Tecnis IOL in distance vision. In near vision, to the contrary. The conclusion is that when the distance vision improves the near vision worsens and vice-versa.

### 310

**Evaluation of the performance of multifocal intraocular lenses image quality: analysis of the standard methods**

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**Purpose** To analyze the standard methods used to describe intraocular lenses (IOL) and to suggest some improvements to make them applicable for multifocal IOLs.

**Methods** The EN ISO standards and the standards of the American National Standards Institute (ANSI) for the evaluation of the performance of IOLs are tested for multifocal IOLs. The MTF of the one refractive multifocal IOL and two hybrid refractive-diffractive multifocal IOLs is measured for both distance focus and near focus and using different pupil sizes.

**Results** The average modulation (AM) from 0 to 100 c/mm of multifocal IOLs (for the distance focus) is about 40 ± 50% lower than the AM of monofocal IOLs, for all pupil sizes studied. This fact suggests that the EN ISO standards must be revised in some points to be adapted for multifocal IOLs. Moreover, the near focus of the refractive IOL only appear beyond 3.5 mm of pupil size, so consequence this type of multifocal IOL, could not be described only with a 3 mm pupil as stated in ISO standard. The width at half height of the point spread function (PSF) or the cut-off frequency value of the MTF are not useful for multifocal IOLs. Multifocal IOLs can have a narrow PSF at half band but be very wide at the bottom, occurring this even in some so-called apodized IOLs. The cut-off frequency value is also useful in monofocal lenses as quality factor to compare MTFs of different lenses or pupil sizes because an approximately uniform slope for the MTF is suppose.

**Conclusion** EN ISO and ANSI, demand the modulation of only one pupil and one frequency: 3 mm and 100 c/mm. We recommend to measure at least with 2 different pupils and to give the AM value for both distance and near focus.

### 311

**High Order Aberrations of the eye implanted the Verisyse® iris-claw intraocular lens**

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**Purpose** To investigate the influence of the secondary implantation site of the Verisyse® iris-claw intraocular lens (IOL) on high order aberrations (HOAs) using wavefront analysis in aphakic patients.

**Methods** Twenty aphakic patients (20 eyes) who had complicated phacoemulsification, leaving no capsular support, but good iris support and clear unwounded cornea implanted with the aphakic Verisyse® (AMO) intraocular lens site either implanted retroprockially or over the iris. Wavefront aberrations were measured using the IR3 Hartmann-Shack aberrometer at 4 mm pupil aperture diameter.

**Results** Nine patients were implanted in the anterior chamber versus 11 who had the IOL clipped behind the iris. Best corrected visual acuity was significantly higher and HOAs were significantly lower in the retroimplanted group.

**Conclusion** In addition to being astigmatic, the Verisyse® intraocular lens implanted behind the iris may restore vision in the absence of capsular support in a more physiologically way than when fixedated over the iris.

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**Secondary IOL implantation without capsular support. A laser flare cell-meter study**

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**Purpose** The cataract surgery consists in phacoemulsification of the lens and implant of an intraocular lens (IOL). In some cases when the capsular support is absent we must use an anterior-chamber IOL (ACIOL) or a posterior-chamber IOL (PCIOL) transclerally sutured. We studied the intraocular inflammation after secondary implantation of iris-fixed ACIOL, angle-supported anterior-chamber (AC)IOL and transcleral-fixed PCIOL.

**Methods** We enrolled 60 patients with post-surgery aphakia, for a total of 60 eyes. Twenty patients received an ACIOL (Artisan), in twenty patients was implanted PCIOL (PC279V) and in the last twenty patients was implanted an ACIOL. Twenty patients with PCIOL in the bag were used as control group. We performed an evaluation of anterior chamber cellularity with a laser flare-cell meter (Kowa 500) 30 and 90 days after secondary implants.

**Results** The anterior chamber flare-cell, after 30 days, was 15,442 ± 10,435 photons/ms in ACIOL group, 22,716 ± 6,634 photons/ms in PCIOL group and 14,11 ± 7,087 photons/ms in the (AC)IOL group. (ACIOL vs PCIOL p=0.001, PCIOL vs (AC)IOL p=0.001, ACIOL vs (AC)IOL p=0.176). After 90 days the flare was 10,937 ± 7,393 ACIOL group, 18,311 ± 6,033 PCIOL group and 10,665 ± 3,959 in (AC)IOL group. (ACIOL vs PCIOL p=0.001, PCIOL vs (AC)IOL p=0.001, ACIOL vs (AC)IOL p=0.5). 76%

**Conclusion** Our study shows chronic subclinical inflammation after 30 and 90 days from implantation of all IOL types but the flare is less in the ACIOLS and (AC)IOL groups than in the PCIOL group. So we think that in without capsular support it is better to implant an anterior chamber iris-fixed or an angle-supported anterior chamber IOL.
Pseudophakic dysphotopsia associated with the implantation of intraocular lenses

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Purpose To determine the incidence of unwanted light images after cataract surgery and the relationship between various intraocular lens (IOL) types.

Methods Prospective study included 50 patients who filled up a written questionnaire where reported on incidence of glare, light sensitivity and unwanted images. Patients were included in the study only if they had uneventful cataract surgery and no additional ocular pathology. They were excluded if they had refractive errors which could cause similar phenomena. The study was performed in three month postoperative period.

Results A significant number of patients reported symptoms. Patients with acrylic IOL were at increased risk. At last control more than 1/3 of symptoms resolved spontaneously. No one reported negative dysphotopsia and there was no IOL exchange.

Conclusion Intraocular lenses can introduce stray light artifacts into the eye. While positive dysphotopsia has been largely attributed to edge effects of the implant, negative dysphotopsia appears to be more related to the patient’s anatomical structure than to specific lens designs or materials.

Anterior chamber depth changes and complications after Nd:YAG laser treatment for posterior capsule opacification in pseudophakic eyes

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Purpose To investigate the effect of Nd:YAG capsulotomy on anterior chamber depth changes and complications of Nd:YAG laser treatment for posterior capsule opacification in pseudophakic eyes.

Methods Our study included 26 eyes (22 patients) with posterior capsule opacification (PCO) after uncomplicated phacoemulsification surgery and intraocular lens implantation. Complete ocular examinations were performed for each patient. The best corrected visual acuity, intraocular pressure, keratometry and anterior chamber depth measurements were obtained in all examinations. In all patients Nd:YAG capsulotomy was performed. Data were analyzed statistically.

Results Mean patient age was 53.73±13.53 years. Before Nd:YAG capsulotomy mean anterior chamber depth was 4.03±0.58 mm and in the first day after capsulotomy mean value was 4.02±0.46 mm. Mean spherical equivalent refraction before laser treatment was -0.53 diopters. Mean spherical equivalent refraction in the first day after laser treatment was -0.49 diopters. Before Nd:YAG capsulotomy mean best corrected visual acuity was 0.38±0.13, and in the first day after capsulotomy mean value was 0.93±0.11, there was statistically significant difference. There were no statistically significant differences between the anterior chamber depth, intraocular pressure (IOP) obtained at before laser capsulotomy and the post laser first day, first month and the third month exams.

Conclusion Nd:YAG laser capsulotomy is an effective and safe method of treatment of posterior capsule opacification.

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**317 Elastic modulus of the iris with drug stimulation**

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Purpose Intraoperative Hippy Syndrome (IHS) syndrome occurs during cataract surgery in patients using Homax. In order to understand iris stiffening with different drugs we investigated how the elastic modulus (i.e., the stiffness) of the iris changes following stimulation by pilocarpine, phenylephrine, and tropicamide.

Methods Irids (n = 20) were dissected from porcine eyes within 4 hr post mortem and mounted on a mechanical testing system. The samples were stretched up to 30% strain in the radial direction, and the modulus was calculated from the linear portion of the stress-strain curve. One of the three drugs of interest was then added (80 µg/ml) to the bath surrounding the tissue and the experiment was repeated. Experiments were performed on intact irides and on strips cut from the iris.

Results Changes in pupil diameter of free-floating samples and in isometric force of mounted samples confirmed that the tissue was responsive to the drugs. For the strips, the untreated iris modulus was 4.0 ± 0.9 kPa (mean ± s.d., n = 20), and the treated iris modulus was 7.7 ± 2.0 (pilocarpine, n = 7), 6.9 ± 2.2 (phenylephrine, n = 6), and 8.4 ± 1.7 (tropicamide, n = 7). Whole irides (n = 10 total) gave similar trends but values were approximately 25% higher: presumably because the shape of the intact iris makes the stress and strain fields inhomogeneous within the sample.

Conclusion Although pilocarpine, phenylephrine, and tropicamide work by different mechanisms, all three gave similar results – an increase in modulus of roughly two times. We conclude that in most normal situations, the iris stiffness varies within about a factor of two range, remaining quite compliant at all pupil diameters. How to address the problem of IHS warrants further research.

**318 Ocular conservation in patients with uveal melanoma by a multimodality approach to treatment**

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Purpose To analyse eye survival in patients with uveal melanoma with a multimodality approach to treatment

Methods 273 patients with uveal melanoma diagnosed at Ocular Oncology Unit of the University Hospital of Valladolid from 1997 September to 2007 April. Pearsonis Chi square test was used to identify between variables and primary enucleation. Logistic regression was used to identify independent variables predicting primary enucleation. Cox’s univariate proportional hazards model was used to identify associations between variables and time to secondary enucleation. Kaplan-Meier estimates were used to draw survival curves for time to secondary enucleation.

Results 273 patients were included in the study. Primary enucleation was performed in 80 patients. Secondary enucleation in 12. uveo-retinal Glaucoma (p=0.0022), tumor diameter ≤5mm (p=0.001), tumour weight >10mm (p=0.001), anterior tumour margin (p=0.001) and extracocular spread (p=0.001) were associated with primary enucleation. Predictive factors for primary enucleation were largest basal tumor diameter (odds ratio [OR], 3.8; 95% confidence interval [IC], 1.5-9.3) and tumour weight (OR, 3.7; IC, 1.7-7.5). Ocular conservation probability 5 years after conservative treatment was 88%. Largest basal tumor diameter, anterior tumour margin and extracocular spread had influence in ocular survival after conservative treatment. Only largest basal tumor diameter was a predictive factor of secondary enucleation.

Conclusion In the present series 69.5% of patients had a conservactive treatment and 88% of them conserved treated eye 5 years.
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The effect of Blue-Light exposure in an ocular melanoma animal model

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**Purpose**

Uveal melanoma (UM) cells lines, when exposed to blue light in vitro, show a significant increase in proliferation. In order to see if the same effect would be seen in vivo, we investigated the influence of blue-light exposure in an animal model of UM.

**Methods**

Twenty albino rabbits were injected with 1 million human UM cells (92.1) into the suprachoroidal space of the eye. Animals were immunosuppressed using cyclosporin A throughout the 8 week long experiment. The animals were divided into two groups of 10 animals each. The experimental group was exposed to blue light, while the control group was protected from blue light using a yellow filter. Intraocular tumor growth was evaluated weekly by funduscopic examination. One animal was sacrificed per week, after the 2nd week, in order to evaluate progression of the disease. The eyes were removed after sacrifice and the proliferation rates of the re-cultured cells obtained from the intraocular tumors were performed using the Sallfordahane8 assay. The Student’s t-test was used to compare results for statistical significance.

**Results**

Re-cultures of UM cells were established from 4 and 5 rabbits of the experimental and control group respectively. Cells were re-cultured for a very short period of time (1 passage only) in order to maintain any in vivo cellular changes. The exposure of rabbits to blue light led to an increase in proliferation of the cell lines derived from intraocular tumors compared to the control (p<0.001).

**Conclusion**

There is increasing amount of data suggesting that Blue light, one of the components of visible light, may influence the progression of UM. Our results warrant further studies to relate the importance of Blue-Light filtering lenses for UM patients.

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AML with bilateral retinal detachment

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**Purpose**

To present a case of thrombotic ocular and CNS involvement complicating acute myeloid leukaemia (AML).

**Methods**

A 42 year old woman developed blurred vision shortly after diagnosis and treatment of M6 AML. Investigations showed anterior orbital infiltraion, retinal detachment and panuveitis. Iris biopsy and vitreous aspirate were negative. She developed right temporal lobe infarction and died following further CNS infarction two months after initial diagnosis.

**Results**

Post mortem examination showed cerebral oedema, multiple cerebral infarctions and heparinomenogly. Both eyes contained vitreus exudates, retinal detachment and uveal thickening. Microscopy showed exudative and haemorrhagic retinal detachment, without inflammatory or neoplastic infiltrate, and bilateral uveal leukaemic infiltration with infarction. Neoplastic cells infiltrated the leptomeninges and brain parenchyma with focal vascular occlusion. Lung vessels were occluded by neoplastic cells. The spleen and bone marrow were heavily infiltrated. Partial immunophenotyping suggested a diagnosis of acute promyelocytic leukaemia (APL).

**Conclusion**

Acute leukaemia involves the eye occurs in 39-53% cases. Visual loss is uncommon. Retinal involvement most frequently occurs in the form of superficial haemorrhages, detachment is uncommon. Acute lymphoblastic leukaemia (ALL) treated with L-asparaginase, acute promyelocytic leukaemia (APL) and non-M3 AML may present with a prothrombotic state which may be catastrophic, as occurred in this fatal case.

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Non Hodgkin orbital lymphoma

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**Purpose**

To present three cases of orbital lymphoma

**Methods**

Detailed ophthalmological exam, laboratory, neuroimaging (the orbital echocan and MR of the brain and orbit), patologist and immunohistochemistry of tumor tissue biopsy. 

Conclusions: The most common cause of secondary glaucoma in RTB is iridocyclitis, followed by papillary block and tumor seeding in the anterior chamber. Aim of this study is to review the glaucoma-induced mechanism in a large population of RTB patients and discuss our experience with these cases.

**Results**

In the last five years we observed 152 cases of RTB; in 18 cases (17%) during the first examination under general anesthesia, elevated intraocular pressure was observed. Histopathological examination of these eyes was performed to understand the mechanisms inducing glaucoma.

**Conclusion**

Although glaucoma is a secondary clinical issue in RTB management and care of its presence, revealed by a thorough oculax exam of the anterior segment, can guide the clinician in assessing the overall condition of the affected eye.
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Autofluorescence and histological findings in cancer-associated retinopathy caused by small cell lung carcinoma

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**Purpose**
To present clinical and histological features of lipofuscin autofluorescence of the retinal pigmented epithelium-RPE in a patient with cancer associated retinopathy-CARC, a paraneoplastic syndrome caused by small cell lung carcinoma SCLC.

**Methods**
87 years old man with SCLC suffered bilateral blindness. Autofluorescence imaging was done using Topcon fundus camera and postmortem light microscopy with autofluorescence and electron microscopy of the retina were done. Retinal photography, autofluorescence angiography, electrophysiology and auto-antibody assay were also performed in vivo.

**Results**
Autofluorescence imaging showed diffuse hyperfluorescence suggesting accumulation of lipofuscin in the RPE and few hypofluorescent atrophic regions due to age-related changes. Autofluorescence microscopy showed lipofuscin accumulation in the RPE and electron microscopy revealed predominance of lipofuscin granules over melanin pigment. Western blot analysis was positive for anti-recoverin antibodies.

The electroretinogram and visual evoked potentials were normal. Histology showed no detection of light microscopy showed complete loss of photoreceptor layer and outer nuclear layer as well as decrease in number of nuclei in the inner nuclear layer with no inflammation. Fluorescence angiography revealed severe arteriolar sclerosis with no occlusions and patchy atrophic changes with no leakage.

**Conclusion**
In CAR, in vivo autofluorescence imaging showed diffuse hyperfluorescence suggesting increased phagocytosis activity and lipofuscin accumulation due to autophagy of the destroyed photoreceptors in the RPE by autoantibody-induced apoptosis, which was confirmed ex vivo by autofluorescence of lipofuscin in histological sections and by electron microscopy.

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Management of dumbbell-shaped dermoid cysts in the temporal fossa and orbit

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**Purpose**
Orbital dermoid cyst occur most frequently besides the lateral orbital wall. Rarely they also extend thorough the frontonasal ostium to the temporal fossa. Here we describe two cases of dumbbell-shaped dermoid cysts and their surgical treatment, from a larger group of epidermoids of the temporal fossa.

**Methods**
Retrospective research.

**Results**
Diagnosis and treatment process is shown.

**Conclusion**
Dermoid cysts are benign teratomas, filled with fluid, granulomatous tissue or fat. Excision is indicated when they give irritation in the surrounding tissues. It is important to think about the extension to the temporal fossa, when treating dermoids of the orbit.

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Oncocytic adenocarcinoma of lacrimal gland with GCDFP15 positivity

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**Purpose**
We present a very rare case of an oncocytic adenocarcinoma of the lacrimal gland. In our case it seems to arise from an oncocytoma and there is an interesting immunophenotype of this tumour, since it appears positive for the GCDFP 15 protein, which has not been described before.

**Methods**
A 77 years old woman presented to the ophthalmologist, complaining of a six month history of pain at the left canthus. Incisional biopsy showed an epithelial oncocytic neoplasm which was reviewed in a tertiary ophthalmic pathology centre, where a immunohistochemistry revealed positivity for CK7 and GCDFP15 (PIP), with negativity for ORE, CK20, HepPar1, AFP, CA19-9, TTF, S100, chromogranin and synaptophysin.

**Results**
After excluding the possibility of metastasis from a non-ocular/adenal primary neoplasm, the underwent left orbital exenteration. Pathological examination showed an aggressive oncocytic adenocarcinoma involving the lacrimal gland and containing a small focus of what appeared to be oncocytoma. The immunophenotype was similar to the original biopsy. Electron microscopy confirmed large numbers of mitochondria present within the tumour cells.

**Conclusion**
We reviewed the ophthalmic literature for previous reported oncocytic adenocarcinomas. Our case is different in that there is a very interesting immunohistochemical profile with a strong positivity for gross cystic disease fluid protein 15 (GCDFP15), which has never been described before in oncocytic lesions of the lacrimal gland. An interesting theory would correlate the fact that both organs (breast and lacrimal gland) derive from ectoderm, but this correlation needs to be thoroughly investigated.

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Silent squamous cell carcinoma invaded into the orbit via the perineural space of the zygomatictemporal nerve

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**Purpose**
Describing two patients who presented with a silent skin tumour invaded into the orbit via an unusual route.

**Methods**
Retrospective case report. We present two patients, one with an immunosuppressive medical history, the other without a relevant history.

**Results**
Two patients present with a tumour which appears to be a tear gland carcinoma. After histology of the biopsies in both patients a squamous cell carcinoma was found. Reviewing original pathology taken two years before admission taken from the patients because of (benign) skin tumours of the temporal side of the orbit demonstrated invasive squamous cell carcinoma. Analysis of possible invasion routes no skin or bone involvement was found neither on CT or MRI. But the lateral fossa near the entrance of the zygomatic-temporal channel showed very small tumours. The course of the zygomatic-temporal nerve is exactly in line with the original skin tumour, the channel and the orbital tumours. The extension of the tumour was in both cases up to the infraorbital fossae, so radical surgery was an illusion. We gave extended radiotherapy involving the cavernous sinus.

**Conclusion**
We found a special route of invading the orbit by a common skin tumour using the course of the zygomatic-temporal nerve. The time elapsed between removing the original tumour and the extension in the orbit took more than two years.
Use of Mitomycin C in the treatment of conjunctival and corneal squamous cell carcinoma. a case report

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Purpose: Conjunctival squamous cell carcinoma is a rare form of slowly progressive carcinoma of low malignant potential. Mitomycin C (MMC) is a chemotherapeutic antibiotic isolated from Streptomyces Cepaciaus and medical literature reports efficacy of MMC in the treatment of squamous cell carcinoma. We present a case of conjunctival and corneal squamous cell carcinoma which was treated with both surgical excision and topical use of MMC 0.02%.

Methods: A 78-year-old man with a red-grey vascularized elevated epithelial lesion that started at the conjunctiva and extended past the corneoscleral limbus on the cornea for about 1.5 mm. The patient was given surgical excision of the conjunctival neoplasia and the histological exam highlighted a corneal conjunctival squamous cell carcinoma. After three months a recurrence of the neoplasia was displayed and we therefore decided to start topical MMC 0.02% eye drops treatment four times daily for 7 days. Four cycles of treatment were done with a one-week interval during which only sodium hyaluronate 0.4% lubricant eye drops were administered.

Results: During the period of treatment with the MMC 0.02% eye drops, the patient did not display any adverse systemic or ocular reactions. At the end of the treatment we found a complete regression of the disease. Twelve months after treatment he was without conjunctival and corneal epithelial dysplasia.

Conclusion: This case highlights the efficacy of MMC 0.02% eye drops in the treatment of conjunctival and corneal squamous cell carcinoma that can avoid the topical use with sponges of MMC at higher concentration that it would present a not predictable long term effects on the conjunctival fornix and on lachrymal secretions.

A new heavy internal taponade in vitrectoreal surgery: an in vitro study

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Purpose: To study the solubility of perfluorohexylciane (F6H8) in silicone oil (PDMS 1000 - polydimethylsiloxane 1000) and to measure the viscosity and the specific gravity of this mixture.

Methods: The solubility diagram of the mixture was obtained with the turbidmetric method, indicating the miscibility of F6H8 and silicone oil 1000 at all the useful temperatures. The viscosity was measured in steady shear conditions by using a controlled stress rheometer (Haake RS150) and a double cone/plate (DC 60/4) system, both at 25 and 37°C. For different volume percent compositions of the mixture. The specific gravity was measured at 37° using a digital densimeter.

Results: A mixture of F6H8 <30% and PDMS 70% v/v was found to be transparent and stable at all the useful temperatures. By combining these proportions of the two substances a resultant density of 1.06 g/cm^3 was obtained. The viscosity of the 30% F6H8 mixture was 203 mPas at 25°C and 163 mPas at 37°C respectively.

Conclusion: The ideal F6H8 and silicon oil mixture can be obtained combining 30% of F6H8 with 70% of silicone oil 1000.
The assessment of retinal layer thickness and volume segmentation reproducibility of optical coherence tomography images

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Purpose Optical coherence tomography (OCT) enables high resolution, cross-sectional imaging of the retina. The various retinal layers can be distinguished on OCT images using an algorithm of our own design. In the present study we aimed to assess the intraobserver variability of the segmentation algorithm.

Methods Ten eyes of five healthy subjects were involved in the study (mean age 29 years). Standard macular mapping was performed using a Stratus OCT device. All OCT images were segmented using an algorithm of our own design, average thickness and volume data were obtained for the following cellular layers: retinal nerve fiber layer (RNFL), ganglion cell layer and internal plexiform layer complex (GCL-IPL), inner nuclear layer (INL), outer plexiform layer (OPL) and outer nuclear layer (ONL). A manual correction tool was developed to overwrite the automatic segmentation in particular cases where the algorithm failed. The segmentation of the same OCT scans was repeated after one week, and the Coefficient of Repeatability (CR) was calculated in order to assess the accuracy of all measurements.

Results The CR results for the thickness of the various cellular layers of the retina (in the order as they appeared above) were as follows: 0.07µm, 0.17µm, 0.50µm, 0.27µm, and 0.28µm. Similarly, the CR for the volumetric measurements were 0.054mm³, 0.027mm³, 0.017mm³, 0.015mm³, 0.014mm³, and 0.025mm³, respectively.

Conclusion The volumetric and thickness results for the various cellular layers of the retina obtained with our custom segmentation algorithm are highly reproducible. According to our results the measurement accuracy of our algorithm is below the resolution of the Stratus OCT device.

Commercial interest

Segmentation analysis of the age related retinal changes in patients with achromatopsia

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Purpose Our previous results showed a significant reduction in the retinal thickness (RT) in the macular region of patients with congenital achromatopsia. In this study we investigated the age dependent changes of the different layers of the central retina.

Methods The retina of eight patients with achromatopsia were examined by optical coherence tomography (OCT). Beside the total retina, the thickness of the retinal nerve fibre layer (RNFL), the inner (IN) and outer retina (OuR), and the pigment epithelium – choriocapillar complex (RPE-CC) was analyzed. Measurements were taken in the fovea (RPE-CC and INr only), at the parafocal rim, and 3 mm away from the fovea using A-scan analysis and callipers. The results of the “younger” (N=5; mean age: 10 yrs, ±2.8) and “older” group (N=3; mean age: 56 yrs, ±15) patients were compared using Mann-Whitney-U test. The analysis was also performed on eight age matched control subjects.

Results The INr and RNFL was significantly thinner (p=0.01; p=0.05) in the “older” compared to the “younger” group in all regions. The OuR and RPE-CC was also thiner in the “younger” group, but these changes were not significant (p=0.05). In the healthy control group only non-significant changes were seen, mainly in the RPE-CC layer. No significant changes were found between the two methods of the measurements.

Conclusion These findings support our theory that the age dependent changes seen in the retinal morphology of the achromatopsia patients are due to the involvement of the cone-related structures rather than the photoreceptors themselves.

The grey fovea sign of foveal edema or serous detachment on non-stereoscopic digital red-free fundus photographs

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Purpose To describe a simple, visually recognizable sign of foveal fluid accumulation on non-stereoscopic fundus photographs recorded in red free illumination and displayed as a grey-scale image.

Methods 100 selected digital fundus photographs from 100 eyes with diabetic retinopathy or central serous chorioretinopathy or from healthy volunteers were graded by 4 masked observers and the result compared using transfoveal optical coherence tomograms as the reference method for determining whether an abnormally thick fovea or subfoveal fluid was present. The photographs were graded for the presence of a darkly pigmented fovea, diffusely circumcised toward the surrounding macula or the absence of this feature; as may be seen when foveal thickening or detachment is sufficient to increase light scatter in the neurosensory retina, making it acquire a shade of grey comparable to the surrounding retina (the grey fovea sign).

Results Mean foveal thickness, including subretinal fluid, was significantly higher in eyes graded as showing evidence of the grey fovea sign (309.3 ± 4.0µm) than in eyes with a dark fovea (282.5 ± 6.2 µm; p < 0.05).

Conclusion The results support the hypothesis that visual grading of grey scale fundus photography in red free illumination enables detection of signs of foveal edema or detachment in the form of a generalized or localized replacement of the healthy dark fovea with a brighter shade of fundus reflection that is nearly identical to the surrounding macula.

The analysis of fundus autofluorescence patterns in retinal diseases

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Purpose Fundus autofluorescence (AF) imaging is a method that shows accumulation of lipofuscin in the retinal pigment epithelium cells in vivo. Fundus AF may be recorded in retinal diseases either by scanning laser ophthalmoscope or by fundus camera using the appropriate filter. The aim of this study was to analyze the AF pattern by both methods.

Methods 20 patients with different retinal diseases including retinitis pigmentosa, cone-rod dystrophy, Stargardt disease. Best macular dystrophy, central serous retinopathy and age-related macular degeneration were included in the study. AF images were obtained from each subject using a confocal scanning laser ophthalmoscope and digital fundus Image camera. The distribution and amount of AF were compared by the use of both systems.

Results All disease entities both instruments showed distinct pattern of AF typical for the disease. Areas of high intensity of AF recorded with HEA matched to areas of increased intensity of AF detected with fundus camera. The distribution of areas of low or absent AF also corresponded well in both systems. Images taken with conventional fundus camera were in general lower contrasted and therefore less sharp. This was particularly true for patients with even mild media opacity. The advantage of fundus camera was however a recording of AF of a greater field of view.

Conclusion AF imaging is a very useful noninvasive method for detecting RPE abnormalities. In clinical practice, when scanning laser ophthalmoscope for recording of AF is not available, conventional digital fundus camera can be used for screening of patients suspected to have retinal disease. Care should be taken in patients with nuclear cataract, as the AF image is influenced by the AF of the crystalline lens by a great amount.
### 337 Ocular and systemic factors associated with diabetes mellitus in the adult population in rural and urban China.
The Beijing eye study

**Poster session 2 : Lens/Cataract - Pathology/Oncology - Retina/Vitreous**

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**Purpose** To assess ocular and systemic factors associated with diabetes mellitus in the adult population in rural and urban China.

**Methods** The Beijing Eye Study 2006, a population based, cross-sectional cohort study, included 3251 subjects (participation rate: 73.2%). Blood samples were available for 2773 (85.3%) subjects. Diabetes mellitus was defined by a fasting plasma glucose concentration >7.0 mmol/L or by a self reported history of diagnosis of diabetes.

**Results** Diabetes mellitus was found in 334 (12%) subjects. In binary regression analysis, presence of diabetes mellitus was significantly associated with higher age, body mass index, systolic blood pressure, triglyceride concentrations, and intraocular pressure, and lower high density lipoprotein level. It was not statistically associated with corneal or subcapsular cataract, size of the optic disc, rim and alpha zone and beta zone, retinal arteriolar arteriolar retinal arterial and venous diameters, retinal microvascular abnormalities, refractive errors and prevalence of glaucoma and early or late stage of age-related macular degeneration.

**Conclusion** In a population-based setting, diabetes mellitus was not associated with optic disc, rim and peripapillary atrophy measurements, retinal vessel diameters and microvascular abnormalities, and prevalence of age-related macular degeneration. Although diabetes mellitus was significantly correlated with higher intraocular pressure, it was not associated with glaucoma.

### 338 Ultrastructure of diabetic epimacular tissue

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**Purpose** Nowadays there exist some different modalities of diabetic cystoid macular oedema (DME) therapy. Authors compare results in laser coagulation against pars plana vitrectomy.

**Methods** 28 eyes of patients with DME were randomized into two groups. One was treated only by laser focal coagulation the seconds only by pars plana vitrectomy with epimacular tissue removal without laser coagulation. Samples of epiretinal tissue from vitrectomized eyes were examined by transmissive electron microscopy (TEM).

**Results** Median duration of DME at the time of PPV was approximately 18.0 (12 - 24) months. The median preoperative best-corrected VA of 0.08 improved to a median postoperative VA of 0.25. 10 eyes without laser coagulation had a median VA improvement of 0.77%, while 10 eyes with macular laser treatment had a median VA improvement of 1.48%. In all 20 eyes, DME was no longer visible on microscopic examination after a median period of 3.0 months. TEM epiretinal tissues samples demonstrate solid blocks of fibroblasts with firm skeleton from collagen fibres.

**Conclusion** PPV resulted in the resolution of oedema, with an improvement in visual acuity in the majority of cases. A randomized controlled prospective trial of PPV versus laser is needed to determine the role of PPV as treatment modality for DME.

### 339 Correlation between fluorescein angiography and optical coherence tomography in diabetic macular edema

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**Purpose** To compare the pattern of fluorescein angiography with the optical coherence tomography in diabetic macular edema.

**Methods** 145 eyes (92 patients) with diabetic macular edema were included. The fluorescein angiography pattern was organized in focal, diffuse, cystoid + focal, cystoid, tractional and detachment of the neuroepithelium. Retinal thickness was measured by optical coherence tomography. Correlation between optical coherence tomography and fluorescein angiography were analyzed.

**Results** Focal type was found in 93.7% of patients of 35.8% and diffuse in 3.44% of the cases. The optical coherence tomography revealed swelling component in 73.28%, cystoid in 41.78%, detachment of the neuroepithelium in 11.8% and tractional in 6.2% of the patients. The swelling image was observed in 78.1% of the patients with focal leakage, 80% of the diffuse and 73.2% of the focal + cystoid. The cystoid component in the 72.3% of the patients with focal + cystoid leakage and 40% of the diffuse + cystoid. The detachment of the neuroepithelium was associated with cystoid swelling in 70.58% of the cases and the tractional detachment with swelling type in 77.7%. None of the serious detachment could be detected with angiography fluorescein.

**Conclusion** The fluorescein angiography do not correlate in all cases with OCT. Macular retinal thickness measurements were higher in the patients with detachment of the neuroepithelium pattern than in the other types of optical coherence tomography features.

### 340 The sensitivity of OCT in the diagnosis of clinically significant macular edema

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**Purpose** To optimize the sensitivity of OCT in the diagnosis of clinically significant macularedema (CSME) in patients with diabetes mellitus.

**Methods** The retinal thickness (RT) of patients with diabetes mellitus and a clinical diagnosis of CSME based on biomicroscopy was measured with the time-domain Stratus OCT and the spectral-domain Topcon OCT. We defined RT as pathological if (1) the difference was 2 standard deviation above the mean RT, using the normative database provided by Zeiss (standard approach) or (2) local abnormalities were seen on the Stratus OCT topographic map optimized with additional scanlines, an adjusted false color scale and 3D representation or (3) the spectral domain OCT 3D and B-scan images showed intra-retinal fluid.

**Results** In total 30 patients were analyzed. Using the standard approach, OCT missed the diagnosis of CSME in 20 patients. On the optimized Stratus OCT topographic map the diagnosis CSME was missed in 9 patients. When looking for intra-retinal fluid on the spectral domain OCT 3D and B-scan images, OCT missed the diagnosis CSME in 7 patients.

**Conclusion** Even after the optimization of the StratusOCT topographic map or with the spectral domain OCT there was a difference in diagnosis of CSME between OCT and biomicroscopy. This difference in diagnosis could be explained by false-positives based on biomicroscopy or false-negatives based on OCT. Future studies are needed to evaluate the clinical relevance of this difference.
**# 341**
A preliminary study of cell therapy of diabetic retinopathy in mice with experimentally-induced diabetes

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**Purpose** To study an effectiveness of xenotransplantation of retinal cells and β-cells of pancreas in mice DBA/2 line with streptozotocin induced diabetic retinopathy

**Methods** 150 male mice (average age: 70 days, weight: 30 g) were studied as experimental and 30 - as control group. For the therapy cells of transgenic MCP rabbit (2.3 passage) were used. Mice were intramuscularly injected 50 μl with 50,000 cells.

**Results** The histological studies showed a positive dynamics in retinal condition in the a group with intramuscular injection of neonatal rabbit's retinal cells without blood sugar level compensation. In two experimental groups (therapy only with β-cells and combined intramuscular injection of the retinal and β-cells) 4 days after the treatment with β-cells of neonatal rabbit's pancreas both groups no clinical symptoms of diabetes (glaucoma, polyuria, polydipsia) were revealed, and blood sugar level was 4.6 mmol/l. All animals became more active than before the treatment.

**Conclusion** The obtained data show that xenotransplantation of retinal cells combined with β-cells of pancreas is more effective for the treatment of diabetic retinopathy than only retinal cells.

**# 342**
Validity of non-mydriatic cameras for screening and follow-up in diabetic retinopathy

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**Purpose** To determine the validity of a non-mydriatic camera for screening and grading diabetic retinopathy (DR). To establish the number of photographs and the field width needed for a correct DR follow-up.

**Methods** A cross-sectional, observational study was carried out to assess the validity of the non-mydriatic Topcon TRC-NW6S retinograph. Validity proportions were calculated. Kappa analysis was made to determine the agreement with conventional fundoscopy exploration performed by indirect ophthalmoscopy and retinal biomicroscopy. One 45° single-field non-mydriatic digital photograph was taken in 82 eyes for DR screening. For DR grading, several combinations of retinal fields were photographed in 247 eyes, first without pupillary dilatation and later with mydriasis.

**Results** In DR screening, 88.2% sensitivity and 96.9% specificity were obtained, where 9% of the tests were invalid. In DR grading diagnosis, the kappa analysis showed close agreement (κ=0.8) based on at least two 45° photographs with mydriasis. However, when attempting to detect macular edema (ME), the maximum kappa statistic obtained did not go above 0.71, showing 67% maximum sensitivity. The sensitivity for detecting derivable DR was similar to that obtained with indirect ophthalmoscopy (94%-98%).

**Conclusion** The non-mydriatic retinograph is a valid instrument for DR screening only when taking one 45° non-mydriatic photograph per eye. However, given that the sensitivity for proliferative DR (PDR) was worse, when grading DR, we would recommend obtaining nine retinal photographs (mosaic) with mydriasis. Used in this way, the apparatus is extremely useful for detecting derivable DR cases.

**# 343**
Posterior sub-tenon triamcinolone acetonide for the treatment of diabetic macular edema

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**Purpose** To evaluate the use of posterior sub-tenon triamcinolone acetonide (PSTA) injection on clinical, angiographic, and optical coherence tomographic (OCT) parameters in diabetic macular edema (DME).

**Methods** In a prospective clinical study, 36 eyes of 33 patients with DME, and a decrease in visual acuity were included. Posterior sub-tenon injection of 40 mg of TA was given under topic anesthesia. All patients were evaluated at baseline and at 1 day, 1, 2, 4, 6, and 8 weeks, and 3, 6, and 9 months after injection. In some of the patients injections were repeated after 3 months. Complete ophthalmologic examination, fluorescein angiography, and OCT were performed before intervention and after 3 months. The main outcome measures were visual acuity (VA), central macular thickness (CMT), intraocular pressure (IOP), cataract progression, and frequency of complications.

**Results** The mean baseline CMT for all eyes was 543μ. The mean CMT was 301μ at 1 month. Twenty-one of the 36 treated eyes showed visual improvement in at least two lines of visual acuity. Nine eyes showed no improvement in vision. Most of them already had poor visual acuity (0.2 or less) before the injections. Complications of the treatment included cataract in three eyes, glaucoma in one. Injection of repository corticosteroids into the posterior sub-Tenon space is of value in the treatment of CME secondary to uveitis. However, we have to beware of the complications of treatment.

**Conclusion** PST injection of TA is effective in reversing CME and improving visual acuity in DME.

**# 344**
Association of single nucleotide polymorphisms (SNPs) of VEGF gene with diabetic retinopathy

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**Purpose** Vascular endothelial growth factor (VEGF) has an important role in the development of diabetic retinopathy. High concentration of vitreous VEGF and elevated VEGF expression has been found in diabetic retinopathy. Single nucleotide polymorphisms (SNPs) of VEGF gene have been shown to influence expression of VEGF. We studied the association of VEGF SNPs with severity of diabetic retinopathy.

**Methods** The study population consisted of 129 diabetic (type 1 or type 2) patients with laser-treated retinopathy, 99 diabetic patients with mild or no retinopathy and 524 non-diabetic controls. SNPs were chosen on structural basis at three different haploblocks of the VEGF gene covering the whole VEGF gene and in addition, polymorphisms previously known to affect VEGF production were selected. VEGF SNPs rs699947, rs2100963, rs2146323, rs3025033 and rs3025039 were genotyped using the 5' nuclease assay for allelic discrimination (TaqMan). The allele and genotype frequencies were analyzed with chi square test in SPSS.

**Results** Duration of diabetes was similar in patients with laser-treated retinopathy and in patients with mild or no retinopathy (mean 22.8 vs. 22.7 years, respectively), but the laser-treated diabetic group had somewhat poorer glycemic control (9.2 vs. 8.4 %). The distribution of genotypes and the allele frequencies did not differ significantly between the diabetics and controls, between patients with laser-treated retinopathy and patients with mild or no retinopathy, or between patients with proliferative retinopathy and patients with non-proliferative retinopathy.

**Conclusion** In conclusion, these findings suggest that VEGF gene SNPs studied are not associated with the severity of diabetic retinopathy.
* 345 Intravitreal Triamcinolone Acetonide to control the diabetic macular edema

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**Purpose** To assess the efficacy of intravitreal Triamcinolone Acetonide (TA) in diffuse diabetic macular edema (DFM) refractory to laser coagulation

**Methods** Descriptive, retrospective, non-randomized study without control group done on 35 eyes of 27 patients with DME & negative dexamethasone test, treated with 4mg intravitreal TA between January 2006 and March 2007. Variation in Visual Acuity (VA)/Snellen Chart was considered as main outcome, and angiographic (AFG) edema was considered as secondary outcome. The statistical analysis was performed by contingency tables, Chi square test and Fisher exact test

**Results** The median basal VA was 0.16, observing an improvement of two or more lines in 35.4% at first month post op and 34.3% at the end of follow up (7 months). Although the edema was resolved in 42.9% at the first month by AFG, it persisted in 77.1% by the end of the study. A mayor effect was observed in patients treated with insulin-oral hypoglycemic diet (p=0.02), cystic CSME (p=0.018), or previous grill laser (p=0.055). A statistically significant relation was observed between VA and the absent of edema in the AFG. Three eyes developed ocular hypertension during the first 24 hours and 4 eyes at first month, all them controlled by topical treatment. No endophthalmitis was observed

**Conclusion** Results were similar to bibliography. A strong relation was observed between VA and AFG during entire follow up. Systemic treatment with diet-insulin oral hypoglycemic agents seemed to induce a better respond to the intravitreal TA in the control of DME

* 346 Retinal layer thickness changes in eyes with diffuse diabetic macular edema

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**Purpose** The leading cause of visual impairment among patients with diabetes is diabetic macular edema, which has several patterns. In our study we aimed to assess which retinal layers show thickness changes in diffuse diabetic macular edema (DDME)

**Methods** 11 eyes with background retinopathy and DDME on optical coherence tomography (OCT) examination and 8 control healthy eyes were enrolled in our study with 1.0 best corrected visual acuity (mean age 54 [37-67] years and 53 [36-67] years, respectively). Standard macular mapping was performed in all eyes using a Stratus OCT™ device. All OCT images were segmented using an algorithm of our own design, average thickness data were obtained for the following cellular layers: retinal nerve fiber layer (RNFL), ganglion cell layer and internal plexiform layer complex (GCL-IPL), inner nuclear layer (INL), outer plexiform layer (OPL) and outer nuclear layer (ONL)

**Results** Average layer thickness significantly increased in all layers in the DDME group except for the RNFL. GCL-IPL: 73.99±10.04 vs. 75.80±3.42 [p=0.006], INL: 34.31±1.90 vs. 37.55±1.39 [p=0.003], OPL: 34.47±2.25 vs. 37.40±2.01 [p=0.013], ONL: 83.48±8.43 vs. 94.67±11.65 [p=0.025], results are mean±SD)

**Conclusion** Our results show that all cellular layers of the retina except for the RNFL are thicker in eyes with diffuse diabetic macular edema. It remains an open question how various cellular layers of the retina are involved in retinal thickening in the case of diffuse diabetic macular edema.

**Commercial interest**

* 347 IL-8 vitreous levels in proliferative diabetic retinopathy

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**Purpose** To determine the levels of interleukin 8 (IL-8) in the vitreous of patients with proliferative diabetic retinopathy (PDR) and the role of IL-8 as a marker of visual prognosis after vitrectomy

**Methods** Vitreous fluid samples were obtained at vitrectomy surgery from 71 patients with diabetes type 2 and PDR, and from 17 age-matched non-diabetic patients with a macular hole (control group). PDR was classified as active and inactive, and subdivided according to the extent of large vessel glotic obliteration. The cytokine levels were measured by Cytometric Bead Array method. To determine the role of IL-8 as visual prognostic marker after vitrectomy we investigated whether the vitreous levels of IL-8 were associated with poor visual outcome. Poor outcome was defined by visual acuity less than 20/20 or at least 20/80 in months after vitrectomy. Clinical and preoperative eye characteristics (visual acuity, iris neovascularisation, vitreous hemorrhage, macular detachment, macular edema, active neovascularisation, neovascularisation of the disk, and the presence of panretinal photocoagulation) were additionally analyzed

**Results** The vitreous levels of IL-8 were significantly higher in patients with PDR in comparison to the control (P<0.001), in eyes with higher extent of large vessel glotic obliteration (P<0.001) and was not significantly higher in eyes with active neovascularisation (P<0.9). After multiple logistic regression analysis, vitreous level of IL-8 (P=0.026) and macular detachment (P=0.039) were predictors for the poor visual outcome after vitrectomy

**Conclusion** The vitreous level of IL-8 was associated with the higher extent of large vessel glotic obliteration and with the poor visual outcome after vitrectomy

* 348 Fundus autofluorescence pattern as risk factor for development of geographic atrophy in patients with age-related maculopathy

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**Purpose** This subgroup analysis within the prospective multicenter natural history FAM study (Fundus Autofluorescence in age-related Macular Degeneration) investigates the tendency of different fundus autofluorescence patterns (FAF) to convert into geographic atrophy (GA). Only eyes with drusen at baseline were observed

**Methods** In addition to ophthalmologic standard examination we took FAF images using a confocal scanning laser ophthalmoscope (HRA, Heidelberg Engineering). FAF was classified into eight different FAF patterns according to the “international fundus autofluorescence classification group” (IFAG).

**Results** 131 eyes of 131 patients with drusen were observed. Incidence rate of FAF patterns was as follows: minimal change in 29 (22%), focal in 28 (21%), patchy in 26 (20%), reticular in 14 (11%), lace-like in 11 (8%), speckled in 7 (5%), focal plaque-like in 3 (3%), and linear in 4 eyes (3%). During follow-up 20 eyes (15%) developed GA. Initially 8 eyes showed lace-like pattern (73% of all eyes with lace-like pattern). Three eyes with focal plaque-like pattern and 3 eyes with linear pattern developed GA (73% of all eyes showing those patterns). Speckled pattern led in 3 eyes (43%) showing this pattern to GA. Two eyes with patchy pattern turned into GA (8%). One eye with focal pattern progressed to GA (4%).

**Conclusion** Lace-like, linear, and focal plaque-like FAF patterns showed the highest risk for progression to GA. At the same time these patterns are relatively rare among all observed eyes with drusen. FAF as a non-invasive examination method and different FAF patterns are usable tools to give a predictive interpretation concerning the risk of a progression from drusen to GA.
Training improves the identification of Retinal Angiomaticous Proliferation in the UK network of ophthalmic reading centres

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Purpose To report on the identification of Retinal Angiomaticous Proliferation (RAP) in the Network of Ophthalmic Reading Centres UK (NetwORC) for the Verteporfin Photodynamic Therapy (VPDT) cohort study. RAP tends to be bilateral and although it is accepted to be part of the spectrum of age-related macular degeneration (AMD), no treatment has yet proved to be as successful as in other types of AMD. A diagnosis of RAP is therefore instrumental to the success of treatment decisions to be made.

Methods The VPDT study involves the grading of more than 5000 angiograms per annum at the network of Reading Centres (RC) with regular training and concordance activities being performed. The grading database was queried for RAP lesions identified on a monthly basis between Nov, 2005 and Oct 2006. Expert training on identification of RAP was given at 3 successive meetings during this period.

Results In this 12-month period, 5402 angiograms were graded (including both primary and quality assurance gradings). RAP was identified in 264 cases, with a step-wise increase in the identification of RAP during this one year period. Using a simple chi squared for trend test, there is a highly significant increasing trend over the 12 months (p=0.0001) following training sessions at all three RCs, highlighting the benefit of structured training.

Conclusion The present study has shown that consistent concordance and training exercises across the RC network improves the identification of specific lesion components such as RAP. With improved education, other clinical and grading staff can be trained to recognize RAP lesions and better identification may lead to improved treatment outcomes.

Glycoproteins of drusen and drusen-like lesions

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Purpose Drusen are a marker of age-related macular degeneration. Lesions similar to drusen, both in histology and their clinical appearance are also seen in choroidal tumours, chronic inflammatory and degenerative conditions of the eye, and in mesangiocapillary glomerulonephritis type II (MCGN-II). This study aims to compare the saccharide composition of these drusen-like lesions in the various ocular pathological groups and in MCGN-II.

Methods Formalin fixed and paraffin was embedded tissue from 21 eyes was studied. The histological diagnoses included AMD, retinal detachment, malignant melanoma, long-standing uveitis, glaucoma and MCGN-II. Glycosylation was examined using a panel of twenty biotinylated lectins and an avidin-peroxidase-DAB-cobalt revealing system, with and without neuraminidase pre-treatment.

Results High mannose, bi/tri-nonbiected and biected complex N-glycan, N-acetyl glucosaminyl, galactosyl and sialyl residues were found to be expressed by drusen, while treatment with neuraminidase exposed subterminal N-acetyl galactosamine and galactosyl residues. Similar binding patterns were found in the various pathological groups studied.

Conclusion As there was no significant difference in the lectin binding pattern in drusen in different pathologies, a common pathogenesis or at least a final common pathway for the elaboration of carbohydrate components of drusen is suggested.

Grading for progression of Age Related Macular Degeneration in the London AMD Study

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Purpose The aim of this study was to assess interobserver variability when grading progression of Age Related Macular Degeneration (AMD) using the International Classification (IC) system and to assess agreement with clinical decision.

Methods Fifty eyes with a minimum of 3 years disease duration documented by colour photographs were selected from the London AMD Study. Changes related to early and late AMD were graded in a random fashion while masked to the main purpose of the study (FS) and then independently by another grader (IL). Images in time sequence were independently analysed for clinical changes. Digitized time-sequence fundus images were then aligned in uniform fashion, arranged into multi-layered composites using Adobe Photoshop and clinical assessment made (TP, ACB). Results were compared and a consensus decision was made.

Results Inter-observer agreement was 89.4% for predominant phenotype (κ=0.84), 89.36%-91.49% for CNV (κ=0.79-0.83), 87.2%-89.36% for GA (κ=0.62-0.74) and 55.32% for area covered by drusen (κ=0.31). In 4 cases the clinical and IC classifications differed drusen area in 2 and two of end-stage disease grading; resulting in 97.8% concordance in identification of change. At baseline, 34 eyes had soft drusen, 15 had CNV and one had GA. Of the 34 with Drusen, 14 developed CNV and 9 GA by the end of the study. Disappearance of drusen without end-stage disease was recorded in two eyes.

Conclusion Overall, changes observed clinically were reflected accurately by the IC grading in the majority of cases. This provides a quantitative basis for using this classification system in longitudinal studies. The accidental observation of regressing drusen without end-stage disease merits further investigation.

The role of telemedicine in improving the referral service for consideration of treatment for age-related macular degeneration in a tertiary referral centre

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Purpose The delay caused by manual referrals of patients from peripheral units for the treatment of age-related macular degeneration resulted in a 15 letter drop in vision making patients ineligible for treatment. We therefore sought to investigate if electronic patient image referrals would reduce this delay.

Methods A customized software package enables the referral centres to securely upload onto the web based solution the encrypted referral packages holding patient demographics in a template and associated colour and fluorescein images. These packages were then safely submitted from the networked computer to a dedicated web server at Moorfields Eye Hospital (MEH). The central site (MEH) then had the ability to import and unpack each package into the telemedicine software programme that triggered an email to the referring centre confirming receipt of the data. Information packages were placed in queues in workflow software which is reviewed daily. As the patient passed through the system the software package triggered the appropriate letter according to the plan of management. This process terminated once the patient had been accepted or rejected by MEH.

Results The beta version of the software fulfills the design specifications of being able to package the complete fluorescein run and colour images from the main camera manufacturers. The software meets the required NHS security standards. Images can be acquired by the treating centre (MEH) the same day as taken in the local referring centre.

Conclusion Telemedicine referrals have been shown to reduce the referral time to treatment while still maintaining a high degree of sensitivity and specificity in identifying the presence of CNV.

Commercial interest
# 353
## Risk factors for age related maculopathy in a Tunisian population

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**Purpose**
The aim of this study is to identify risk factors for AMD in the Tunisian population which is exposed to intense sunlight and have important seafood diet.

**Methods**
A prospective study was conducted including 2204 persons aged more than 50 years which came to our hospital as patient or as accompanying adult. Patients responded to a questionnaire asking about medical history, habits and behaviour, sun exposure, diet and smoking habits. A complete ophthalmic examination was undertaken in patients with AMD. The median age of patients was 66 ± 9.5 years old (range 50-100). Lesions are classified in early and late stages of AMD. Statistical analysis was done.

**Results**
Prevalence of AMD in our studied population was 16.4%. Age was a significant risk factor. Prevalence of AMD was 12.5% in group patient aged between 50 and 60 years and 31% in group patient aged more than 80 years (P=0.001). Males were more exposed to AMD than females (P=0.001). Excessive sun exposure, lack of nutrients and antioxidants increased significantly the risk of AMD (P=0.10). Smoking was the most important risk factor (P=0.10) and prevalence increased proportionally to the importance of cumulative annual consumption. Cardiovascular diseases, diabetes and dyslipidemia were not significantly associated to a high prevalence of AMD. Cataract extraction did not increase the risk of AMD in our study and myopia was associated to a low risk of AMD.

**Conclusion**
AMD is a multifactorial disease. Some preventive measure were effective. Genetic studies of the disease could identify population at risk.

# 355
## Intravitreal bevacizumab to treat subfoveal choroidal neovascularization in highly myopic eyes

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**Purpose**
To describe the anatomical and visual outcome of subfoveal and juxtapfoveal choroidal neovascularization (CNV) in highly myopic (HM) eyes treated with intravitreal bevacizumab.

**Methods**
Prospective, non randomized, multicentric, interventional case series. 17 highly myopic eyes from 16 patients with subfoveal or juxtapfoveal choroidal neovascularization were treated by three monthly intravitreal injections with 1.25 mg bevacizumab. Patients were evaluated for best corrected visual acuity (BCVA) and optical coherence tomography (OCT) at baseline and monthly before the injections were performed. Fluorescein angiography (FA) was performed at baseline and at month 3.

**Results**
Patients averaged 48 ± 3 years of age (SD 15.3; range 29 to 72). Five patients were males and 11 were females. All eyes were followed for six months or longer. BCVA at baseline averaged 20/67 (range 20/160 to 20/32) (63.9 letters, SD 10, range 40 to 75) and 20/48 (range 20/125 to 20/69) (66 letters, SD 12, range 45 to 85) at the end of follow-up (p=0.001 and p=0.002 respectively). Average central foveal thickness was (CFT) 253.5 microns (SD 40.1, range 205 to 340) at baseline and 202.1 microns (SD 31.7, range 154 to 251) at the end (p=0.001). Leakage from CNV ceased in all eyes at month three. No occult or systemic safety issues appeared during follow-up.

**Conclusion**
Intravitreal bevacizumab seems to be an effective and safe therapeutic procedure to treat subfoveal or juxtapfoveal CNV in HM eyes. Further studies are required to verify the efficacy and usefulness of this therapy compared with established treatments for CNV in HM eyes.

# 356
## Full field ERG values before and after anti-VEGF treatment in AMD affected subjects

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**Purpose**
To compare ERG b-wave amplitude differences before and after treatment with sodium pegaptanib intra-vitreal injection.

**Methods**
14 patients all affected by exudative AMD were submitted to scotopic, combined and fotopic full field ERG, to estimate photoreceptorial electric activity through b-wave amplitude. All patients have been then treated with 1 to 4 intravitreal injections of 0.3 ml sodium pegaptanib. Time between each injection was 2-4 weeks. After one and two months than first injection all patients were again submitted to full field ERG. Data were related with ERG obtained before first injection.

**Results**
In 6 patients we found a b-wave amplitude increase of about 20% two months after first injection. Remaining 8 patients showed no valuable differences in ERG amplitude before and after treatment.

**Conclusion**
In a valuable number of subjects, sodium pegaptanib intravitreal injection, seem to improve photoreceptorial electric activity. Other studies are needed to confirm this hypothesis.

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**Effects of sodium pegaptanib intravitreal therapy in subjects affected by wet age-related macular degeneration not-responsive to photodynamic therapy: angiographic, OCT and microperimetric data**


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**Purpose**
To evaluate the effects of antiangiogenic treatment with sodium pegaptanib intravitreal injections in subjects with wet age related macular degeneration (AMD) already treated with verteporfin photodynamic therapy (PDT) and reactivation of choroidal neovascularization (CNV)

**Methods**
6 eyes with CNV due to AMD previously treated with PDT that showed new vessels and leakage at FA, were treated with an anti-VEGF, pegaptanib sodium. We performed 3 intravitreal injections (each 0.3 mg of drug) at distance of 6 weeks, a biweekly analaysis by fundus-related micropimeter and OCT, and a monthly examination by FA.

**Results**
We observed in all patients a reduction of membrane size and leakage at FA, a reduction of membrane thickness at OCT and an increase of retinal sensitivity at microperimetric analyses. The treatment for CNV due to AMD with PDT is directed to the neovascularizations that proliferate and leak blood and fluid. The mean number of treatments to obtain a therapeutic success with this therapy is 5.4 in two years. However sometimes it is not efficacious. The anti-angiogenic agents target key mediators of the angiogenic cascade that leads to the development of neovascularization. This different mechanism of action can be useful in those patients not responsive to PDT.

**Conclusion**
The effects of antiangiogenic treatment with pegaptanib sodium intravitreal injections in subjects with wet AMD already treated with verteporfin PDT and reactivation of CNV are reduction of size, leakage and thickness of membrane and also an increase of retinal sensitivity.
# 357
Effects of autologous translocation of the choroid and retinal pigment epithelium on the retinal light response in patients with neovascular age-related macular degeneration

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Purpose The aim of the present study was to evaluate potential electrophysiological changes after autologous translocation of the choroid and retinal pigment epithelium for subfoveal choroidal neovascularisation in patients with age-related macular degeneration.

Methods A consecutive series of 20 patients suffering from subfoveal choroidal neovascularisation secondary to age-related macular degeneration underwent autologous translocation of the choroid and the retinal pigment epithelium. The ERG was recorded one day prior to the translocation surgery and no earlier than 4 weeks after the silicone oil removal.

Results The ERG amplitudes were significantly reduced after translocation surgery. The mean b-wave amplitude reduction of the scotopic ERGs was 36.1%. The a- and b-waves of the saturating light response decreased significantly by 42.9% and 41.1%, respectively. The photopic a- and b-wave amplitudes were significantly lower after the translocation surgery resulting in a mean reduction of 23.7% and 40.1%, respectively. Furthermore, the mean amplitude of the flicker ERG showed a mean decrease by 26.2%.

Conclusion The present study indicates that a significant electrophysiological decrease is caused by autologous translocation of the choroid and retinal pigment epithelium. However, this surgical procedure proved to substantially reduce the neuroretinal trauma when compared with the ERG data of historical controls that underwent 360° retinotomy and macular translocation.

# 358
A case of lead toxicity and associated maculopathy

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Purpose To present a case of a 50 year old man who presented with reduced visual acuity in his left eye following a history of occupational lead poisoning dating back 3 years.

Methods The initial presentation was in March 2006 when the visual acuity in the right eye was 6/9 and the left CE. There was a left RAPD. The anterior segment examination and IOP were normal. The left macula showed pigmentary epithelial changes similar to a Bull’s Eye type maculopathy. The right eye had a granular appearance at the macula.

Results MRI scans performed were normal and the VF result for the right eye was noncontributory. Electrodiagnostic testing showed a left sided optic nerve dysfunction but well preserved ERGs with no interocular difference. FFA showed a window defect at the site of the pigment epithelium atrophy. OCT demonstrated a cystic space at the level of the outer segment of the photoreceptors in the fovea. Tests for potential inflammatory, infective and congenital causes for unilateral optic neuritis found no abnormality.

Conclusion The case provides evidence of lead toxicity being the cause of unilateral optic neuritis and RPE changes leading to significant visual impairment.

# 359
Optical coherence tomography in patients with cone-rod dystrophy

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Purpose To analyze morphologic characteristic features in patients with cone-rod dystrophy (CORD) and examine correlation between visual function and morpohmetric parameters.

Methods 8 patients (aged 16-33, mean 22 yrs) with the clinical diagnosis of CORD underwent OCT (Stratus OCT 3, Zeiss) examination. Foveal thickness (FT) and total macular volume (TMV) were evaluated. Disturbance of central visual acuity was detected at the age of 6-7 yrs. Photoversion is a common clinical symptom.

Results FT of patients ranged 96-182 micrometers (mean 139.75 +/- 26.1), TMV ranged 46.75 mm³ (mean 56.1 +/- 9.94). FT and TMV of the normal cohort in our Institute range between 130-180 micrometers and 6.2-7.2 mm³ both parameters of the patients showed a decrease of the lower limit compared to the normal values. Alterations observed in the foveal architecture were retinal thinning and blunting of the foveal pit. There was only a weak positive correlation (r=0.443) between visual acuity and foveal thickness, and it was moderately stronger (r=0.527) between visual acuity and total macular volume.

Conclusion OCT is a useful non-invasive additional method in the differential diagnosis of cone-rod dystrophy.

# 360
Foveal damage volume and visual acuity in solar retinopathy

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Purpose To correlate the size of foveal lesion measured by OCT and the visual acuity in patients complain of acute solar retinopathy.

Methods We studied patients diagnosed of acute solar retinopathy after a solar eclipse by OCT. In the acute phase, all the patients showed a hyperreflective lesion in the fovea using OCT. We measured the volumes in pixels of this foveal lesion by software image analysis (MatLab 7) and correlated this value with the best corrected visual acuity (Snellen). To evaluate the correlation we used the Spearman test and a level of P<0.05 was accepted as statistically significant.

Results Eighteen eyes were enrolled in the study. Mean age was 32.15±12 years and 33.3% were woman and 66.7% were man. Mean best corrected visual acuity was 0.65±0.29. In 100% of the eyes was observed a hyperreflective foveal lesion using OCT. Mean pixel volume lesion was 6997.62±4662.229. The correlation (r) of foveal lesion volume and best corrected visual acuity was 0.793 (p<0.05).

Conclusion Solar retinopathy shows a typical image in OCT, a hyperreflective lesion located in the fovea. We report a negative relation between the size of the above mentioned injury and the visual acuity. Thus, the OCT can be of great help for the diagnosis of this disease and the evaluation of the foveal damage.
361 Results of optical coherent tomography in children with myopia
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Purpose During the last five years the works which were conducted to find peculiarities of myopic eyes with the help of optical coherent tomography (OCT) appeared. Authors in patients without ophthalmoscopic changes in the retina found signs of dystrophy by OCT in different layers such as in pigment epithelium, retinal fiber layer (RNFL) in central and peripapillary segments of retina (Adel M. Hassan & al., 2004 Liu H.D. & al., 2006, Mrugacz M. & al., 2005). Thickness and volume of macular retina depends on its axial length (Lim M.C., 2005) but some data are controversial. The investigation performed to reveal early signs of myopia progression in retinal anatomy -in live- in patients with school myopia without visual changes in the eye fundus.

Methods The comparison of macular thickness, retinal nerve fiber layer thickness and optic nerve parameters were evaluated in 21 child (42 eyes) with ‘school myopia’ of light (1.0–30 diopter) and high (6.0–7.0 diopter) degree (18 and 24 eyes accordingly), which were observed with the help of Stratus OCT Model 3000 Carl Zeiss Meditec, Inc.

Results Average macular volume and thickness was almost equal in both groups of children, values of peripapillary RNFL thickness were lower (82, 8 ± 13.7 against 95.3 ± 12.4), and Cup/Disc Area ratio (0.1-0.3) decreased in children with myopia of high degree in comparison with children with light degree (0.7-0.9).

Conclusion It is obvious that children have to be observed for a period to establish if above mentioned parameters: peripapillary RNFL, Cup/Disc Area ratio are useful to predict myopia progression.

362 Scleroplasty with posterior pole buckling for the treatment of high myopia
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Purpose The main pathogenetic factor of the dystrophic process which develops in macular zone of a highly myopic eye is the extension of shells of the posterior eye pole accompanying the damage of vascular and nervous structures and Bruch’s membrane.

Purpose: to develop and assess clinical results of a simplified technique of scleroplasty combined with the posterior pole buckling using a new implant directed to support the posterior pole and decrease the stress in the eye shells.

Methods Surgery was made by a modified technique of Snyder-Tomson using biologically active polyether buckle sutured to a scleral graft. The buckle was located on the posterior pole, the ends of the graft were fixed with some tension. 14 surgeries were performed on 12 patients aged 13-40 with myopia of 11.0-31.0 D. In the first 5-7 days after surgery, instillations of 0.5% timolol solution were used. The observation period was 3 years. The shape and stage of staphyloma before and after surgery were determined by ophthalmoscopy and using ultrasound B-scan.

Results In the immediate postoperative period, B-scan determined flattening of staphyloma: a shortening of the antero-posterior axis by 1.0–1.5 mm, and an increase in visual acuity by 0.1-0.2. A 5.3 dB increase of posterior pole sclera acoustic density was observed. In the late observation period, staphyloma stabilization was achieved, accompanied by the inhibition of the dystrophic process in the macula.

Conclusion Flattening of staphyloma and shortening of the antero-posterior axis evidently bring about a reduced posterior pole shell stress and a decrease of vitreoretinal tractions. This confirms a favorable effect of the surgery on the biomechanics and tropism of posterior eye pole shells.

363 Photodynamic therapy in myopic eyes with CNV with marked leakage
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Purpose Choroidal neovascularization (CNV) secondary to pathological myopia is an important cause of significant visual impairment in young and middle age globally. To assess the effect of verteporfin photodynamic therapy (PDT) in choroidal neovascularization (CNV) secondary to pathological myopia (PM) with CNV with marked leakage.

Methods We prospectively followed a series of 44 consecutive patients (45 eyes) with photogenic myopia (>5-6 diopters) who received verteporfin PDT for CNV. Particular attention to the detection of leakage for neovascularization. This population was divided into two groups according to leakage of the neovascularization: group A: minimum leakage; group B: marked leakage.

Results The time of follow up was 24 months. (range, 12 to 60 months). Visual acuity (VA) improved by 1 lines in group A and decreased by 2 lines on ETDRS charts in group B. The final mean VA in group B was 66.2 letters and 34.4 letters in group A.

Conclusion Verteporfin PDT has carried at a stable or improved vision in myopic eyes with CNV with marked leakage and appear to respond better to treatment respect to the CNV with minimum leakage.

364 The vasodilatory effect of juxta-arteriolar microinjection of endothelin-A receptor inhibitor in healthy and in acute branch retinal vein occlusion Minipig Retinae
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Purpose To investigate the vasactive effect of endothelin-A receptor inhibitor BQ-123 on the retinal arterio-vascular unit in normal and in acute branch retinal vein occlusion (BRVO) minipig retinae.

Methods Under general anesthesia, 6 normal eyes of 6 minipigs and 5 eyes of 5 minipigs with acute experimental BRVO were evaluated. In all eyes, a slow, continuous microinjection of 30 µl BQ-123 (0.67 µg/ml) was performed 50 to 100 µm away from a retinal arteriole, avoiding exerting pressure on retinal anatomic structures. After the microinjection, the retina were observed for changes in vessels’ diameter. The procedure was recorded in real time. Throughout the procedure, the animals’ vital signs were monitored.

Results Overall (n=11), an increase of 18.56% and 26.45% in arteriolar diameter was evidenced 5 and 15 minutes respectively after BQ-123 microinjection. When separately examining normal (n=6) versus OBRV (n=5) eyes, the increase in arteriolar diameter 5 minutes post injection was 16.75% versus 19.86% respectively (P=0.491), whereas the increase in arteriolar diameter 15 minutes post injection was 25.14% versus 27.4% (P=0.591).

Conclusion Our results demonstrate a significant increase in retinal arteriolar diameter after juxta-arteriolar BQ-123 microinjection, both in healthy and in acute OBRV retinae. There was no statistically significant difference between the amount of vasodilation exerted by BQ-123 on healthy and on BRVO retinae. This vasodilatory effect may be beneficial in counterbalancing or reversing the arteriolar vasoconstriction that settles within hours following BRVO, with subsequent improvement of arteriolar blood flow.
Early treatment favours the anatomical outcome of ROP

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Purpose: A comparative study between two protocols in ROP management

Methods: In this study, participated 1716 premature neonates (SG=32 vs. BW<1500g), divided into two groups: Group A-854 who followed and treated according the CRYO-ROP study guidelines and Group B-862 who followed and treated according to the ET-ROP study guidelines. In both groups cryotherapy were performed when necessary.

Results: In group A, 372 eyes developed some type of ROP; in 46 eyes cryotherapy was performed and in 15 of those (32,6%) appeared with unfavorable anatomical outcome (total or partial retinal detachment & dragged macula). In group B, 358 eyes developed some type of ROP; in 76 eyes cryotherapy was performed and only 3 (3,94%) eyes appeared with unfavorable anatomical outcome after cryotherapy.

Conclusion: Earlier intervention in high risky and closer monitoring in low risky ROP neonates proved very effective in our sample. Since unfavorable events due to general anesthesia appear less often nowadays, early intervention could be proved very successful and safe procedure.

The retinal vessel response to flicker light in patients with central retinal vein occlusion

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Purpose: The study examined the retinal vessel diameter (A/V Ratio) and the response to flicker light in patients with central retinal vein occlusion and in patients with arterial hypertension.

Methods: The prospective study included 30 patients. In the group I were 12 patients with central retinal vein occlusion (CRYO), in group II 10 patients with hypertension and in group III 8 healthy volunteers. A/V ratio (static analysis) and the diameter response of an arterial and venous vessel segment to flicker light (dynamic analysis) were measured with a Retinal Vessel Analyzer. Each dynamic examination consisted of 50 s baseline measurement and three periods of 20 s flicker light provocation followed by 80 s observation. The mean of three flicker cycles was calculated as dynamic vessel response.

Results: Statistically significant differences of A/V ratio were observed in the static vessel analysis between groups I-III. (A/V ratio: 0.71±0.07, 0.76±0.06 and 0.82±0.02 respectively). The dynamic analysis showed statistically significant differences in arterial dilatation and constriction between all investigated groups as well. The venous flicker response showed significant differences between healthy controls (group III) and group I. and II. No significant differences in venous response were measured between group I and II.

Conclusion: The results show significant differences in A/V ratio and arterial response to flicker stimulation in patients with CRYO. We suppose that the degree of retinal vessels endothelial dysfunction is one of the leading factors in the ethopathology of central retinal vein occlusion.

Thrombophlebitis and bilateral visual loss and in Behçet’s disease

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Purpose: To describe an unusual case of Behçet’s disease characterized by bilateral ocular involvement and thrombophlebitis in the right leg.

Methods: A 37-year-old man was referred with a two-month history of progressive, severe visual loss in both eyes. He had had thrombophlebitis in his right leg following an insect bite a month before. Visual acuity was R.E.: light perception and L.E.: 20/400. Fundus examination disclosed bilateral papillitis and retinal vasculitis and a macular pucker in the right eye.

Results: Fluorescein angiography showed occlusive arteritis with peripapillitis in both eyes. TC and MR imaging and laboratory tests for toxoplasmosis, Lyme disease, syphilis, HIV, HSV, and Rickettsia conorii infection were negative. Conversely, ESR, PCR and HLA B51 were found to be positive. Treatment with systemic steroids produced no improvement.

Conclusion: Ocular Behçet’s disease is occasionally not associated with the classic systemic signs, such as oral aphthous lesions, skin lesions, and genital lesions. In this report, the retinal lesions followed an episode of thrombophlebitis caused by an insect bite.

980 nm Diode laser retinochoroidal photocoagulation – in vitro comparison with frequency doubled Nd:YAG (532 nm) laser

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Purpose: To evaluate acute effects of diode laser (980 nm) on retina and choroid and to compare these effects with the effects of frequency doubled Nd:YAG laser (532 nm) on same eye structures.

Methods: Freshly enucleated pig’s eyes were treated with diode laser (980 nm) and frequency doubled Nd:YAG (532 nm) through the slit lamp adaptors. Same energy densities were used. Histological examination of the treated eyes was performed after photocoagulation.

Results: Changes in photoreceptor layer (slightly increased eosinophilia) and outer nuclear layer (slightly condensed nuclear chromatin) of retina and coagulation necrosis of choroid were present in all laser lesions. There were no significant differences in retinal changes produced by both lasers. Coagulation necrosis of choroid was more pronounced in lesions produced by 980 nm diode laser.

Conclusion: Acute effects of both lasers were similar, although thermal damage produced by 980 nm diode laser extended deeper. The 980 nm diode laser could be used for retinochoroidal photocoagulation.
Trace elements in the retina of eyes enucleated due to complications of a severe trauma

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Purpose to find out whether trace element concentration in the retina is related to chronic eye pathologies caused by traumatic eye injuries.

Methods The content of 12 trace elements in the retina (Zn, Cu, Fe, Al, B, Si, Ca, Ba, Ti, Mg, Mn, Pb) was analyzed using an atomic absorption spectrophotometer AS-3 and a Perkin-Elmer chromatoo-mass spectrometer. 25 samples of 25 enucleated eyes of patients aged 17 to 68 (average age 31.6±2.6 years) were studied. Enucleations were required due to complications of a severe trauma: secondary glaucoma, buphthalmus, subatrophy, uveitis, retinal detachment, amauonos, corneal leukemia, or combinations thereof.

Results Retinal samples revealed a significant scatter of trace element concentration: the level of Ca varied from 10 to 1000 mg/kg, Fe, 10 to 350 mg/kg, Mg, 30 to 150 mg/kg, Al, 3 to 70 mg/kg, Si, 0.1 to 50 mg/kg, Mn, 0.3 to 50 mg/kg, Zn, 3.0 to 30 mg/kg, B, 0.03 to 30 mg/kg, Cu, 0.5 to 17 mg/kg, Ba, 0.05 to 6 mg/kg, Ti, 0.05 to 2 mg/kg, Pb, trace quantities to 28 mg/kg. Typical changes of trace element concentration in the retina for different eye pathologies could be revealed. Secondary glaucoma and buphthalmus showed reduced content of Ba, Ti, Al, B, Mn, Zn and Mg and a 5-fold increase in Cu and Si. The opposite changes were found in chronic uveitis: levels of Fe, B, Al and Cu showed a 2 to 4-fold increase while Si showed a 2-fold decrease.

Conclusion The revealed changes may be linked to intravinous violation of trace element metabolism in glaucoma, a shortage of elements participating in the metabolism of photoreceptors and neural structures of the retina was found, whilst uveitis demonstrated an increase of elements linked to the activation of peroxidation.

Effect of intravitreal injection of indocyanine green, triamcinolone acetonide and trypan blue on the electroretinographic response in the rat

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Purpose The purpose of this study was to evaluate the effects of intravitreal injection of ICG (indocyanine green), TB (trypan blue) and TA (triamcinolone acetonide) on the visual function assessed by electroretinogram (ERG) in the rat.

Methods Three groups of 12-week-old Sprague-Dawley rats (n=6) received intravitreal injection in one eye of 0.1ml of either ICG 0.5mg/ml, TB 3mg/ml or TA 40mg/ml, followed by a rinse with 1ml of saline solution. The contralateral eye was used as a control and was injected similarly with saline only. The scotopic ERG was recorded at different intensities (10tdcs/m², 2500tdcs/m² and 25000tdcs/m²) before injection and 28 days after treatment.

Results No effect of the treatment was observed on the ERG amplitudes and wave latencies in control eyes and in eyes treated with TB and TA. The ERG b-wave amplitudes and latencies were significantly reduced in eyes treated with ICG at 10tdcs/m² and 25000tdcs/m² but not at 2500tdcs/m² (amplitude means: t0=174.8µV versus t28=23µV; p<0.05; t0=174.8µV versus t28=23µV; p<0.05; t0=140.3µV versus t28=40.0µV at 25000tdcs/m² p=0.07). The ERG b wave amplitudes and latencies were also significantly lowered at 25000tdcs/m² in eyes treated with ICG (amplitude means: t0=45.6µV versus t28=23µV; p=0.05; latency means: t0=7.6msec versus t28=9.2msec p=0.01).

Conclusion This study shows a side effect of ICG on retina function four weeks after a transient retinal exposure. TA and TB could be considered as interesting alternatives to ICG for macular surgery.
Poster session 3: Neuro-ophthalmology / Strabismology / Paediatric / History, Glaucoma, Physiology/Biochemistry/Pharmacology

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Optic radiation damage can be assessed directly by tractography
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Purpose To verify the clinicoradiological correlation of tractographs of optic radiations (OR) involved by brain tumors and infarcts.

Methods We performed diffusion-tensor magnetic resonance imaging (DT-MRI) on 7 patients with brain lesions adjacent to or involving the visual pathway, 5 of whom underwent DT-MRI before and after surgical excision of the brain lesions. Tractographic findings of OR on coronal reconstruction images, including those in the contralateral hemisphere, were analyzed with respect to their location and size and correlated with existing visual field defects. When the ipsilateral OR appeared to be reduced in cranio-caudal height by >70% or more in coroal section than the contralateral OR, we considered the finding abnormal.

Results The contralateral ORs were successfully depicted in all cases. In 8 examinations the ipsilateral ORs were successfully depicted, in 4 they were not. The 8 depicted ORs in the ipsilateral hemisphere were abnormal either in size (n=8) and/or location (n=4). OR defects were noted in either the superior or inferior portions in 8 tractographs. OR deviation due to mass effect from the brain lesions was noted in 4 tractographs. In 5 tractographs with OR defects there was a good correlation with the location of the visual field defect.

Conclusion The tractography demonstrates the ORs all the time on the contralateral side, correlates some of our cases with the visual field defects on the ipsilateral side.

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Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency in anterior ischemic optic neuropathy
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Purpose The reported incidence of G6PD deficiency in Sardinia, Italy, ranges from 10% to 15%. Evidence indicates that patients with G6PD deficiency are protected against ischemic heart and cerebrovascular disease and retinal vein occlusion. The purpose of this study was to assess the incidence of G6PD deficiency in Sardinian patients with anterior ischemic optic neuropathy (AION) and ascertain whether G6PD deficiency may have a protective effect against these vascular disorders.

Methods G6PD blood levels were measured in 153 patients (70 males, 83 females) with AION. 306 age- and sex-matched subjects undergoing cataract surgery and having no history of AION served as controls. Logistic regression models were used to investigate the association between G6PD deficiency and AION.

Results G6PD deficiency was found in 71 (46.4%) out of 153 patients with AION and in 121 (42.2%) of 306 controls. Differences between AION patients and controls were statistically significant (odds ratio 0.35, 95% confidence interval: 0.15-0.81, P=0.01).

Conclusion The incidence of G6PD deficiency in Sardinian patients with AION was lower than expected. Results suggest that patients with G6PD deficiency have a significantly lower risk of developing AION.

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Diagnosis and follow-up in patients with optic neuritis by means of optical coherence tomography (OCT) and scanning laser polarimetry (GDx)
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Purpose To evaluate the accuracy of oculart imaging technologies, such as Optical coherence tomography (OCT) and Scanning laser ophthalmoscopy (GDx) in the diagnosis and follow-up of optic neuritis.

Methods A total of 133 patients with optic neuritis and 13 age- and sex-matched healthy controls were included in this prospective, clinical study and followed up for six months. All of them underwent neurologic assessment and a complete ophthalmic examination that included visual acuity, color vision ( Ishihara pseudosochromatic plates), visual field, OCT and GDx.

Results In acute episode, there were significant differences in visual acuity, Ishihara colour test, visual field mean deviation (MD), either in anterior or posterior optic neuritis, compared with healthy eyes. Eyes with acute papillitis showed increased RNFL thickness measured by OCT in every retinal quadrant compared with healthy eyes, but differences were not statistically significant. Six months after the acute episode, visual acuity, colour test and visual field had improved but we observed RNFL thinning in eyes with optic neuritis compared with control eyes. Both the OCT and the GDx detected significant differences between the groups six months after the acute episode.

Conclusion Structural analysis of the RNFL by means of OCT and GDx is useful in optic neuritis diagnosis and follow-up. Optic neuritis, either anterior or posterior, causes a decrease in the RNFL thickness, which can be detected by OCT and GDx.

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Ocular motility disorders in a Fabry patient
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Purpose M. Fabry is a rare lysosomal storage disease affecting among other organs the vascular endothelium.

Methods We present a 38 years old male Fabry patient with a prior history of 6 different episodes of stroke. We examined him 3 months after his last stroke in our department for newly developing ocular motility disorders.

Results Slit lamp exam revealed bilateral cornea verticillata, conjunctival vessel anomalies and tortuous retinal vessels. Bilateral blepharospasm pronounced on the right side was present. Bilateral rotatory nystagmus was also noted. MRI showed diffuse white matter lesions. For the blepharospasm botulinum therapy was instituted.

Conclusion Most likely Fabry disease causes cerebral ischemia in an atypical pattern, in our patient affecting among others different nuclei responsible for ocular motility.
**405** Visual function vs. spastic quadriplegia & brain damage
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**Purpose** To investigate whether visual function is related to severity of spastic quadriplegia (SQCP) and brain damage.

**Methods** 52 SQCP children participated in this study. All of them were classified according to Gross Motor Functional Classification System (GMFCS) and also had a brain MRI and an eye testing (visual resolution evaluation and strabismus assessment) using the VFA K test.

**Results** The brain MRI showed that: 18 children appeared with mild to moderate periventricular leukomalacia (PVL), 14 with severe PVL, 3 with cortical atrophy, 13 with combined PVL and cortical atrophy and 4 appeared with parenchymatic atrophy. According to GMFCS classification, 6 appeared with good motor function, 8 with a mild motor problem, 9 with moderate motor problem, 29 with severe to very severe motor disability. In visual testing, 14 children appeared with visual resolution (VR) < 0.23 cpd, 19 children appeared with 0.43 cpd < VR < 0.87 cpd and 19 children appeared with VR > 1.75 cpd. Also, 23 children were orthophoric, 19 esotropic (25-60Δ) and 10 children were exotropic (25-50Δ).

**Conclusion** SQCP with poor GMFCS score, moderate to severe PVL, or with combined PVL and cortical atrophy, appeared with the most affected visual function.

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**Purpose** To present a new test assessing the visual function of cerebral palsied (CP) children. The reliability and validity of the test are also presented.

**Methods** 200 CP children (2 - 16 years old) (145 spastic quadriplegies and 55 spastic diplegics), 90 healthy children (2 - 15 years old) as norms and two doctors as examiners. The reliability and validity of VFA test were evaluated by the intraobserver agreement, test-retest. The VFA test assesses 10 important visual parameters (i) assessment of visual attention, (ii) assessment of visual gratings / acuity for far, (iii) visual acuity for near, (iv) contrast sensitivity, (v) peripheral vision, (vi) colour perception, (vii) binocular status, (viii) execution of saccades, (ix) execution of smooth pursuits, (x) eye-hand coordination), leading to 10 subscores. The sum of these is called Index K, ranging from 0 to 62, and represents the visual ability of each CP child.

**Results** The statistical analysis proved that the VFA K test is a reliable and valid test. Further analyzing our data, we found that 31 CP children (15%) appeared with Index K 0-20, 34 children (17%) with Index K 21-40, 50 children (25%) with Index K 41-50 and 86 children (43%) with Index K > 51. 20.83% of spastic quadriplegies appeared with Index K ≤ 20, 23.61% with 21 ≤ Index K < 40, 27.77% with 41 ≤ Index K < 50 and 27.77% with 51 ≤ Index K ≤ 62. In diplegics subgroup: none of the children appeared with index K < 41, 17.85% appeared with 41 ≤ Index K ≤ 50 and 83.15% with 51 ≤ Index K ≤ 62.

**Conclusion** The VFA-Kozeis test is a reliable, valid, easy to perform, cost and time consuming procedure, assessing the visual function of CP children.

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**407** Could vision prognosis cerebral palsy children’s development?
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**Purpose** To investigate the role of vision as a prognostic factor of the general developmental process in children with cerebral palsy (CP).

**Methods** 80 CP children (age range 24-96 months), participated in the study. Anamnestic data was collected, evaluation of Developmental Quotient (DQ), assessment of visual function (VFA K test), Visual Evoked Potentials (VEPs) and brain magnetic resonance imaging (MRI), were performed in all. VFA K test assesses the visual function in a general way (sensory, oculomotor, eye body coordination).

**Results** 53 CP children (66.25%) appeared with affected visual function and reduced DQ, 75.55% of the CP children with moderate to severe PVL and 93.75% with combined brain damage, appeared with affected visual function. Statistical analysis showed a significant relationship between visual function, DQ and the severity of brain damage. (p < 0.001). On the contrary, only 63.63% of the cases with affected visual function, appeared with abnormal flash VEPs.

**Conclusion** Since visual function shows a statistically significant relationship with the developmental quotient of these children, visual function could play a prognostic role in these children’s general development.

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**408** Visual function vs. spastic diplegia & brain damage
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**Purpose** To investigate whether visual function is related to severity of spastic diplegia (SDCP) and brain damage.

**Methods** 28 SDCP children participated in this study. All of them were classified according to Gross Motor Functional Classification System (GMFCS) and also had a brain MRI and an eye testing (visual resolution evaluation and strabismus assessment), using the VFA K test.

**Results** The brain MRI showed that: 23 children appeared with mild to moderate periventricular leukomalacia (PVL), 2 with severe PVL, none with cortical atrophy, 3 with combined PVL and cortical atrophy and none with parenchymatic atrophy. According to GMFCS classification, 6 appeared with good motor function, 11 with a mild motor problem, 9 with moderate motor problem, 2 with severe motor disability and none with very severe disability. In visual testing, 7 children appeared with 0.85 cpd and 21 children appeared with 1.75 cpd. Also, 24 children were orthophoric, 4 esotropic (25-60Δ) and none was exotropic.

**Conclusion** The visual function is not much affected in the majority of SDCP children, unless GMFCS score and brain damage are significantly affected.
Peculiarities of pupil reactions in patients with hyperopic amblyopia in dependence of monocular fixation

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Purpose: Data of pupil reactions in patients with amblyopia revealed disturbances in pupillary afferent pathway in 9% - 93% cases (Tychsen L., Hoyt W.F.,1985, Portny T.Z., & al.,1983), and disorders of peripheral parts of retina in amblyopic eyes. The aim of the work was to study pupil reactions in patients with hyperopic amblyopia in dependence of monocular fixation and in comparison with emmetropic patients.

Methods: Pupil reactions (direct, sequence and accommodative) in patients with hyperopic amblyopia were studied with elaborated pupillography in 72 children aged 5-14 years old (50 with amblyopia and 22 with emmetropia). Amblyopia of high degree was in 22% of patients, medium and light degree was in 48% and 60% accordingly. Degree of hyperopia in both eyes was (2.14±0.99)dpt; astigmatism was below 2.0 dpt. Central fixation was on 27 and eccentric in 23 cases.

Results: It was established that values of pupil latency recovery after direct flash stimulation were larger than in healthy person, values of active constriction latencies, term of active constriction and recovery latencies during the consequent pupil reaction were different in healthy and amblyopic eyes and degree depends on monocular fixation of amblyopic eye in comparison with healthy persons who had equal values. During accommodative reaction average maximal pupil area values and term of pupil recovery were larger in amblyopic eyes than in eyes of healthy patients; the largest were in cases with eccentric fixation.

Conclusion: It is suggested that not only afferent pupil way function is disturbed as it was known before, but also the efferent ways are involved in amblyopia especially in cases with eccentric fixation.

Dynamic retinal vessel reaction in patients with primary open angle glaucoma

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Purpose: Impaired vascular regulation might contribute to glaucomatous damage. Whether retinal branch arteries and veins of healthy persons and primary open angle glaucoma (POAG) patients show different reactions in response to flickering light stimulation (FLS) is investigated.

Methods: Retinal vessel reactions to FLS were examined in 28 POAG patients (stage I, 54.3±9.9 years old) after 4 week wash-out of eye drops and in 28 age and gender matched medically healthy volunteers. Vessel diameters of retinal vessel segments were assessed by Dynamic Vessel Analyzer (DVA). After baseline measurement (50 s) monochromatic rectangular FLS (520±600 nm, 12.5 Hz, 20 s) was applied.

Results: In most subjects fast vessel dilation compared to baseline and an ensuing reactive arterial constriction were observed. In detail we found:

<table>
<thead>
<tr>
<th>POAG</th>
<th>control</th>
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<tbody>
<tr>
<td>mean arterial dilation at the end of FLS</td>
<td>3.3±2.7</td>
</tr>
<tr>
<td>time of max arterial constriction following FLS</td>
<td>49.9±26.7</td>
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<tr>
<td>mean venous dilation at the end of FLS</td>
<td>2.9±1.9</td>
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We found statistically insignificant differences between the two examined groups as marked with (*p<0.01) (U-test).

Conclusion: Functional retinal arterial and venous dilation in response to FLS does not differ between POAG patients and healthy subjects. Reactive arterial constriction following the FLS appeared later and venous restoration occurred faster in POAG. These findings might be an indication for alterations in the vascular endothelium and vessel wall rigidity in POAG, leading to impaired regulation following metabolic demand.
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Glucoma progression with GDx-VCC

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Purpose To analyse the stability or progression of glaucomatous damage with scanning laser polarimetry (GDx-VCC) in a longitudinal prospective study.

Methods 57 glaucoma patients and 54 healthy volunteers were examined with GDx-VCC and standard automatic perimetry (SAP) in every 6 months. Stability or progression was assessed according to the standard visual field progression criteria. The relationship between the change of the retinal nerve fibre layer thickness (RNFLT) and the elapsed time was examined with linear regression analysis. The baseline SAP parameters were compared with unpaired t-test. For each individual, the data of one eye were used.

Results The follow-up time varied between 18 and 32 months. SAP progression was detectable in 17 glaucomatous eyes. The baseline visual field mean defects of the eyes with stable and progressing glaucoma did not differ significantly (7.144 dB and 9.175 dB, p=0.33). On eyes with progression, a significant negative relationship was detected between Superior Average, Superior Maximum and Maximal Modulation, and time (p<0.05). On healthy eyes and glaucomatous eyes with stable visual field, the time had no effect on the RNFLT parameters (p>0.05).

Conclusion The GDx-VCC may be suitable for detecting mild glaucomatous progression.

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Outcome and risk factors for failure of primary trabeculectomy

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Purpose To evaluate the outcome and risk factors for failure in primary trabeculectomy in glaucoma patients.

Methods Medical record review of 59 patients (66 eyes) who underwent primary trabeculectomy from 2002 to 2006 at the Eye Hospital Ljubljana. Complete success was defined as intraocular pressure (IOP)≤18 mmHg without glaucoma medications, and failure as IOP>18 mmHg with or without medications.

Results The mean preoperative IOP was 31.4 mmHg (±9.7) on an average of 2.5 glaucoma medications, and the mean postoperative IOP after 20 months was 16.8 mmHg (±6.4) on an average of 0.6 medications. Twenty months after trabeculectomy 51 of 66 eyes (77.3%) had an IOP<18 mmHg without medical treatment. Ten of 15 eyes (66%) experienced failure within the 6 months after trabeculectomy. Using Kaplan-Meier life table analysis the 3 and 5 year probability of an IOP<18 mmHg without medication was 70%. After 3 years, the cumulative survival rates for eyes after trabeculectomy with mitomycin C (22 eyes), 5-fluorouracil (31 eyes) and trabeculectomy without antimetabolite (13 eyes) were 78%, 77%, and 57%, respectively.

Postoperative interventions (needling of filtering blebs and subconjunctival injections of 5-fluorouracil) were associated with a higher risk of failure with the hazard ratio of 2.9 (P<0.04). Patients’ age, number of preoperative medications, and cataract surgery were not significant predictive factors for failure.

Conclusion The outcome of primary trabeculectomy in our patients is comparable with that reported in other populations using the same criteria for success. More pronounced healing response after surgery necessitating needling of filtering blebs and subconjunctival injections of antimetabolite are associated with a higher risk for trabeculectomy failure.

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Treatment of patients with late complications after filtering surgery

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Purpose Demonstrate the possibilities of successful surgical treatment of patients with late complications after filtering surgery.

Methods This study shows patients who have developed a cystically dilated or leaking filtering bleb in the late post-surgical period after filtering surgery. Excision of the inadequate filtering bleb has been performed in the patient with a cystic bleb. After the failure of less invasive methods of treatment, we have proceeded to bleb revision with a scleral patch graft in the patient with a leaking bleb.

Results Patients treated with either bleb excision or scleral patch graft showed no signs of neither hypotonia nor secretion during the six-months post-surgical period. During that period, complicated cataracts developed. After cataract surgery the function of the filtering bleb was preserved.

Conclusion During the several months of patients’ follow-up, reconstructive methods of treatment have shown to be successful in high-risk patients who have developed post-surgical complications in the form of leaking or inadequate filtering bleb.

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Ultrasound biomicroscopy in non penetrating filtration surgery with 5-Fluorouracil and without implant: long-term results

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Purpose To evaluate the long-term morphological changes in the anterior segment structures of the eye that appear after non penetrating filtration surgery (NPFS), supplemented with antimetabolites and without implant using ultrasound biomicroscopy (UBM).

Methods 13 eyes of 13 consecutive patients who underwent NPFS with 5-fluorouracil and without collagen implant were evaluated in an observational non-randomised, consecutive case series. The study was conducted at least after 1 year of the surgery. The following parameters were assessed: (1) presence of subconjunctival filtering bleb, (2) presence and volume of an intracocular cavity and (3) presence of a suprachoroidal hypoechogenic area. IOP was evaluated before and after surgery.

Results Mean intraocular pressure (IOP) decreased significantly from 32±7.69 mmHg preoperatively to 13.76±4.1 mmHg at the time of UBM (p<0.01). 69.2% of the patients had POAG≤21mmHg without medications and 84.6% with medication. UBM demonstrated a subconjunctival empty space in 92.3% of the eyes and in 84.6%, an intracocular cavity was observed. The mean volume of this cavity was 1.68 mm³ (range 0-1.25). We found correlation between the height (r=0.610), the width (r=0.754), and the volume of the intrascleral lake (r=0.558). In 92.3% of the eyes a hypoechoic area in the suprachoroidal space was also observed.

Conclusion UBM demonstrated an intrascleral cavity after NPFS without using an implant similar that are described when it is used. At least 1 year after the surgery the majority of the eyes have an intrascleral cavity and a suprachoroidal space.
Trabeculectomy and Mitomycin C (Trab-MMC) for uveitic glaucoma: post surgical interventions

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Purpose To examine the outcome of patients who underwent trabeculectomy and MMC for refractory uveitic glaucoma and determine the post operative interventions required

Methods The Birmingham ReGAE (Research into Glaucoma and Ethnicity) Project is an open, prospective, consecutive series of patients who had undergone trabeculectomy with MMC. The surgery was performed by a single surgical team at the Birmingham and Midland Eye Centre

Results Twenty eyes of 25 patients with uveitic glaucoma underwent Trab-MMC for uncontrolled IOP despite maximally tolerated medical treatment. Mean follow-up was 650 days. Mean pre-op IOP was 26.7 mm Hg and post-op was 11.5 mm Hg. 92 % of trabeculectomies reached complete success (IOP < 21 mm Hg without medication). Subconjunctival injections of 5-fluorouracil were performed on 10 eyes (36 %). Bkble needling revision was performed on 5 eyes (18 %). Visual loss > 2 lines occurred in 1 case (3.8%) due to delayed hypotony maculopathy

Conclusion Trabeculectomy and Mitomycin C is effective in this complex series of patients but do require considerable post surgery manipulation

Intravitreal bevacizumab for neovascular glaucoma

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Purpose To study the short term efficacy of intravitreal bevacizumab in a series of patients with neovascular glaucoma.

Methods Eleven patients with neovascular glaucoma and symptomatic elevation of intraocular pressure were treated with 1.25 mg/0.05 ml of bevacizumab. In three patients intravitreal Avastin was given preoperatively, one day before pars plana vitrectomy. Additional cyclophotocoagulation was performed only if intraocular pressure was not controlled with topical medication. All patients were followed-up for a minimum of 8 weeks.

Results Intravitreal application of bevacizumab resulted in a marked regression of iris neovascularization in all patients in the first three postoperative days. Intraocular pressure was sufficiently controlled in seven patients. Four patients required additional cyclophotocoagulation. We noted no side effects from intravitreal bevacizumab.

Conclusion Intravitreal application of bevacizumab resulted in a marked regression of iris neovascularization in all patients in the first three postoperative days. Intraocular pressure was sufficiently controlled in seven patients. Four patients required additional cyclophotocoagulation. We noted no side effects from intravitreal bevacizumab.

Is nonadherence with eye drop treatment associated with increased intraocular pressure?

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Purpose Effectiveness of clinical glaucoma treatment may be dependent on the patient’s adherence (A) with eye drop treatment. The relation between non-adherence (NA) and intraocular pressure (IOP) is not yet well understood, due to methodological weaknesses in the studies performed so far. The goal of this study was to investigate the association between NA with eye drop treatment, IOP and degree of visual field (VF) defects in a large sample of glaucoma patients.

Methods A multi-center cross-sectional survey was performed in 973 patients in 60 ophthalmologic centers in Belgium. NA was assessed by a written self report question asking patients how many times he/she had forgotten to administer eye drops during the past 2 weeks. Answer options ranged from never, seldom, once a week to daily. The patients indicating they had skipped 1 or more administrations were classified as NA. Current IOP (mm Hg) and VF defects were measured with a Goldmann application tonometer and perimeter, respectively, at the same time as the NA assessment.

Results 603 out of 973 (64%) questionnaires were available for analysis. 39.5% of the patients reported to be NA. NA patients showed a significant higher current IOP of the left eye than A patients (18.12 +/- 3.15 vs 15.58 +/- 2.81; p=0.030). No significant difference in IOP of the right eye was found between NA and A patients (16.15 +/- 3.53 vs 16.02 +/- 7.61; p=0.843); The degree of visual field defects was not significantly related to NA.

Conclusion Because of the difference in IOP of the left eye between A and NA patients, NA should be carefully monitored as part of glaucoma management. Yet, prospective studies are needed to confirm the relationship between NA and poor outcomes.
**# 421**

Adjunctive use of tafluprost with timolol provides additive effects for reduction in intraocular pressure in patients with glaucoma

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**Purpose** Beta-blockers and prostaglandin F2a (PGF2a) analogues reduce intraocular pressure (IOP) by complementary mechanisms. A combination of treatments with additive effects may be beneficial to further reduce IOP in patients not sufficiently controlled with a single agent. This study aimed to investigate the efficacy and safety of tafluprost, a new PGF2a derivative in development, as adjunctive therapy to timolol.

**Methods** In this multi-national, multi-centre, parallel-group, double-masked, phase III study, 185 patients with open-angle glaucoma or ocular hypertension and with IOP between 22.20 mmHg on timolol, were randomised to tafluprost 0.0015% eye drops (n=96) or vehicle (n=89) as adjunctive therapy with timolol 0.5% eye drops for 6 weeks. The primary endpoint was change from baseline in the overall diurnal IOP at 6 weeks. Safety parameters were also analysed.

**Results** At week 6, the additional effect of tafluprost, as compared to timolol baseline, was between 22.24%. This additional effect was consistently larger than in the vehicle group. There were more ocular events, including conjunctival redness, iris colour and eyelash changes with tafluprost compared with the vehicle (overall 42% vs 29%), but most were mild in severity.

**Conclusion** Tafluprost, when administered as adjunctive therapy to timolol, is effective and well-tolerated for the treatment of glaucoma and ocular hypertension.

**Commercial interest**

**# 422**

The IOP-lowering efficacy of once-daily Travoprost /Timolol dosed in the morning compared to twice-daily Dorzolamide /Timolol for patients with open-angle glaucoma or ocular hypertension

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**Purpose** To evaluate the IOP-lowering efficacy of dosing Travoprost 0.004%/Timolol 0.5% Ophthalmic Solution (Trav/ Tim, once-daily morning dosing) and Dorzolamide 2%/ Timolol 0.5% Ophthalmic Solution (Dorz/Tim), dosed twice-daily.

**Methods** This was a 6-week, randomized, double-masked, multi-center, parallel-group study. A total of 319 patients were enrolled, 154 were randomized to Trav/Tim at 9 AM and placebo at 9 PM and 165 were randomized to Dorz/Tim at 9 AM and 9 PM. Efficacy assessments included mean IOP and mean IOP change from baseline at 9AM and 4PM at Week 2 and Week 6. Patients were monitored for adverse events.

**Results** Baseline mean diurnal IOPs were 26.0 mmHg for the Trav/Tim and 26.1 mmHg for the Dorz/Tim group. Mean Week 6 diurnal IOP values were 16.4 mmHg for Trav/Tim and 17.2 mmHg for Dorz/Tim. Trav/Tim produced greater reductions in IOP than Dorz/Tim at all time points. Mean diurnal IOP differences were statistically significant (P=0.05) at 2 weeks, 6 weeks and when the data were pooled over the course of the study. Both medications were well-tolerated.

**Conclusion** Travoprost 0.004%/Timolol 0.5% Ophthalmic Solution dosed once-daily, produced diurnal IOP lowering efficacy that was superior to Dorzolamide 2%/ Timolol Ophthalmic Solution 0.5% dosed twice-daily.

**Commercial interest**

**# 423**

The influence of selective laser trabecuoplasty on diurnal intraocular pressure in POAG

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**Purpose** To investigate the influence of SLT on mean diurnal IOP (mean IOP) and diurnal IOP fluctuation.

**Methods** 26 eyes of 13 POAG patients underwent a 4-week washout before baseline diurnal IOP curve. Standard 30° SLT was performed. IOP was controlled at week 1 and month 1. The IOP curve was repeated after 3 and 6 months. IOP lowering medication was started (failure) if mean IOP was higher than 22 mmHg, or any single IOP value exceeded 26 mmHg at any visit.

**Results** Post-treatment IOP elevation <5 mm Hg was found in 16 eyes (62%). An at least 20% decrease of the mean IOP was not reached. Baseline mean IOP and diurnal IOP fluctuation (mean – SD) were higher for the 11 eyes requiring medication before the month 3 visit than for the other eyes (mean IOP: 28.8±5.2 mm Hg vs. 20.8±4.1 mm Hg, p=0.0011; IOP fluctuation: 9.5±4.2 mm Hg vs. 6.8±1.9 mm Hg, p=0.031). For the eyes with no medication mean IOP changed from 19.3±1.4 mm Hg (baseline) to 18.6±1.4 mm Hg (month 3, Duncan test, p=0.0078) and to 18.2±2.0 mm Hg (month 6, p=0.017). No difference was seen between month 3 and 6 (p=0.351). Diurnal IOP fluctuation decreased from 7.2±2.3 mm Hg (baseline) to 4.3±1.7 mm Hg (month 3, p=0.0004) and to 5.1±1.7 mm Hg (month 6, p=0.0004). No difference was found between the month 3 and the month 6 values (p=0.225).

**Conclusion** SLT resulted in a decrease of the mean diurnal IOP and the diurnal IOP fluctuation.

**# 424**

Nycthemeral rhythm of intraocular pressure in normal tension glaucoma

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**Purpose** To characterize the circadian variations of intraocular pressure (IOP) in the normal tension glaucoma (NTG).

**Methods** Fourteen patients with NTG were studied. The age range was from 45 to 87 years old. All patients underwent a complete ophthalmologic examination (including corneal thickness and visual field (Humphrey type). Then, IOP measurements were hourly controlled by Tonopen TM1, during a 24 hour cycle associated with sleep monitoring. The sleep study included a polysomnography (to exclude a sleep apnoea syndrome) and was associated with 24 hour measurements of blood pressure and heart rate. The nycthemeral rhythm was defined according to the cosine technique.

**Results** Sixty percent of the patients exhibited an absence of circadian rhythm of IOP among 24 hours. Twenty one percent of the patients had a nocturnal rise of IOP and 21% of the patients had a diurnal rise of IOP (<21 mm Hg). A sleep apnoea syndrome was diagnosed in 71% of the patients. Abnormal nocturnal dips of arterial pressure were present in 78%, a vasospastic syndrome was present in 41.6% with and 40% of the patients exhibited an systemic hypertension.

**Conclusion** In our study, most of the patients showed an absence of nycthemeral rhythm of IOP. In this population, a high prevalence of sleep apnoea syndrome, of abnormal nocturnal dips of blood pressure, and of vasospastic syndrome was found.
**425**

**Ophthalmoscopic estimation of cup-to-disc ratio and its relation to the evening intraocular pressure in normal individuals**

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**Purpose** To record and relate the cap-to-disc ratio (C/D ratio), as assessed by ophthalmoscopy and the evening intraocular pressure (IOP) of normal individuals of various age groups.

**Methods** 467 normal individuals, randomly selected, were examined by the same experienced ophthalmologist. The C/D ratio was assessed by ophthalmoscopy and the IOP was measured in the evening by Applanation Tonometry. The individuals were divided into three age groups (Group A: 120 individuals 20-40 years old; Group B: 233 individuals 41-60 years old; Group C: 114 individuals 61-80 years old).

**Results** Group A: C/D ratio ranged from 0 to 0.9 (mean±SD 0.496±0.221) and IOP ranged from 8 to 22 mmHg (mean±SD 13±3.6±26.5mmHg). Group B: C/D ratio ranged from 0 to 0.85 (mean±SD 0.452±0.212) and IOP ranged from 8 to 22 mmHg (mean±SD 14±7.9±27.81mmHg). Group C: C/D ratio ranged from 0 to 0.85 (mean±SD 0.499±0.221) and IOP ranged from 10 to 30 mmHg (mean±SD 14±13±2.419mmHg).

The relation (r) between IOP and C/D ratio was as follows:
- Group A: r = 0.268 (p=0.001), Group B: r = 0.063 (p=0.091), Group C: r = -0.109 (p=0.001).

**Conclusion** The C/D ratio values were, in general, larger in individuals with higher IOP values. However, this relation was weakly statistically significant. In Group B the relation was of lower statistical significance than in the Group A and in the Group C. This weak relation is possibly indicative that other factors except for IOP contribute to the final C/D ratio of normal individuals. There are individuals with high C/D ratios and relatively low IOP values and vice versa. The examiner is responsible for identifying cases which should undergo further investigation.

**427**

**Short-term influence of antiglaucomatous medications on the ocular pulse amplitude (OPA) measured with the dynamic contour tonometer Pascal**

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**Purpose** To assess the short and medium-term influence of antiglaucomatous treatment on the OPA value by DCT.

**Methods** Prospective non-randomized study including 35 eyes of 25 patients (mean age: 63±10.9 years) with newly diagnosed POMG or OHT and treated with latanoprost, timoptoest or Cosopt. The IOP and OPA were measured with GAT and DCT before starting the study and after the 1, 3 and 6 months of treatment. Central corneal thickness (CCT), systolic, diastolic blood pressure (BP) and cardiac frequency were recorded. Pearson coefficient was used to assess correlations.

**Results** The mean GAT IOP was 25.8±5.6 mmHg at baseline, 17.7±3.6 mmHg, 17.9±3.2 mmHg and 17.6±3.3 mmHg at first, 3 and 6 months post-treatment respectively (p<0.001). From a mean baseline IOP of 24.2±3.9 mmHg, the mean DCT IOP was 17.9±2.6 mmHg, 17.6±2.5 mmHg, 17.5±2.3 mmHg at 1, 3 and 6 months (p=0.001). Both mean GAT and DCT were correlated with CCT (p<0.001). GAT and DCT IOP were strongly correlated at all visits. The mean pretreatment OPA was 3.3±0.9 mmHg. It was significantly reduced to 2.6±0.7 mmHg at first month, 2.6±0.7 mmHg at third month and 2.8±0.6 mmHg at 6 month, without difference between the pretreatment visit (regression, p=0.05). The baseline OPA was significantly correlated with DCT IOP, systolic and diastolic BP. It was only correlated with IOP at first month (p=0.05). The decreases in OPA and IOP were significantly correlated.

**Conclusion** Antiglaucomatous medications are associated with a significant short-term decrease in OPA which remains stable in the medium-term.

**426**

**Effectiveness of intraocular pressure phasing**

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**Purpose** To investigate the value of intraocular pressure phasing during normal working hours in patients with and without anti-glaucoma treatment.

**Methods** This study is a retrospective case note review of 61 patients referred for intraocular pressure phasing (every 2 hours from 8.00am to 4.00pm). Comparison between pre-phasing intraocular pressure measurements (mean and range) taken during earlier routine visits (5 measurements) and phasing intraocular pressure (mean and range) for each eye was performed using paired t-test. Scatter plots were used to display the relationship of pre-phasing and phasing intraocular pressure. Subgroup analysis of 119 eyes into untreated and medically treated groups was performed.

**Results** Eyes in the untreated group (51 eyes) showed no difference between the mean intraocular pressure at pre-phasing and phasing (p-value=0.8) but a significant difference between the intraocular pressure range (p-value=0.007). For the medically treated group (68 eyes), there was no significant difference between the mean intraocular pressure at pre-phasing and phasing (p-value=0.9) and no significant difference between the intraocular pressure range (p-value=0.66). In the total 119 eyes the peak intraocular pressure was found to occur at 10.1 am.

**Conclusion** Intraocular pressure phasing is most useful on untreated patients. Intraocular pressure phasing is less likely to give much additional data, compared to pre-phasing intraocular pressure measurements, in treated patients. If a clinician wanted to identify the peak intraocular pressure the best time is between 10-11 am.

**428**

The comparison of the quality score of IOP and ocular pulse amplitude (OPA) values measured by dynamic contour tonometer

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**Purpose** To measure the IOP independently of the corneal properties. It is foreseen of a digital LCD screen which displays the IOP, the OPA, and the quality score (Q) of measurements varying from 1 (excellent) to 5 (poor). The manufacturer recommends discarding the IOP and OPA values with Q4 and 5. The aim of our study was to assess if IOP and OPA measurements with Q3 are acceptable for clinical and research purposes.

**Methods** 2 consecutive GAT IOP measurements were followed after 10 minutes break by 3 consecutive DCT IOP measurements in 216 patients. Only 85 patients who had IOP and OPA values with Q score 1, 2 and 3 were included in this sub-study. The mean DCT IOP and OPA were taken for the statistical analyses. DCT IOP Q3 was separately compared with Q2 and Q1 values. DCT IOP Q1 and Q2 were compared as well. The same comparison was performed for OPA. The Pearson correlation was used for correlation assessment.

**Results** The mean age was 56±14 years. The mean IOP with GAT was 18.2±4.4 mmHg. The mean IOP Q3, Q2 and Q1 were respectively 17.8±3.3, 17.5±3.3 and 17.5±3.4 mmHg. The mean OPA Q3, Q2 and Q1 were respectively 2.5±1.0, 2.5±1.0 and 2.5±0.9 mmHg. The Pearson correlation for DCT IOP Q3-2, DCT IOP Q3-1 and DCT Q2-1 were equal to 0.9 (p=0.001). The Pearson correlation for OPA Q3-2 was 0.8; for OPA Q3-1 0.7 and for OPA Q2-1 0.8. The difference between these values were not statistically significant.

**Conclusion** The IOP and the OPA values with Q 1, 2 or 3 measured by DCT are not significantly different and can be taken into account for clinical and research purposes.
**Dynamic contour tonometry in corneal oedema**

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**Purpose**

Tonometry in corneal oedema is a current problem. In clinical routine intraocular pressure (IOP) may be measured erroneously too low in edematous thickened cornea using Goldmann applanation tonometry. To compare Goldmann applanation tonometry (GAT) and dynamic contour tonometry (DCT; Pascal, Technomed, Germany) in postoperative corneal oedema.

**Methods**

Fifty patients with cataract were included in a prospective study. IOP was measured by means of GAT and DCT before and one day after cataract surgery. Corneal thickness was determined using a Scheimpflug camera system (Pentacam, Oculus, Germany).

**Results**

After surgery corneal thickness increased significantly (pre-surgery: 548 μm, post-surgery: 677 μm; p<0.0001). No significant difference of IOP values measured with DCT compared to GAT was detected before and after cataract surgery (pre-surgery: GAT: 17 ± 5 mmHg, DCT: 17 ± 6 mmHg; post-surgery: GAT 15 ± 7 mmHg, DCT: 15 ± 7 mmHg). IOP measured with DCT and GAT were significantly correlated (pre-surgery: r=0.808, p<0.0001; post-surgery: r=0.767, p<0.0001). The difference between GAT and DCT pre-surgery compared to post-surgery was not significantly different. The IOP difference using GAT or DCT pre-surgery compared to post-surgery was not correlated to the change in corneal thickness.

**Conclusion**

DCT does not give any additional information compared to GAT in patients with corneal oedema. However, a marked difference in IOP values using GAT or DCT is apparent in some subjects.

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**Comparison of DCT, GAT and ORA values in patients with glaucoma**

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**Purpose**

The Ocular Response Analyzer (ORA) is a novel device for the measurement of intraocular pressure (IOP), corneal hysteresis (CH) and the corneal resistance factor (CRF). We compared the ORA with Dynamic Contour Tonometry (DCT) and Goldmann Applanation Tonometry (GAT).

**Methods**

We examined 63 eyes of 33 glaucoma patients (primary open angle glaucoma (POAG) n=26, normal tension glaucoma (NTG) n=10, ocular hypertension (OHT) n=15, pseudoxfoliation glaucoma (PXG) n=12). After assessment of corneal thickness (CCT) by Orbscan, IOP was measured in a randomized order with the ORA, DCT and GAT. For each device mean IOP and for the ORA mean IOP, CH and CRF were calculated and assessed for potential dependency on CCT.

**Results**

Mean IOP in mmHg was 20.6±6.1 SD for the ORA, 18.2±4.2 SD for DCT and 16.5±4.4 SD for GAT. Bland-Altman Analysis showed a good agreement between DCT and GAT, while the ORA showed a tendency to higher values in patients with higher IOP. None of the devices showed a dependency on CCT (GAT: r=0.12, DCT: r=0.07; ORA: r=0.08). There was no correlation between CH and CCT (p=0.081), but there was a weak correlation between CRF and CCT (p=0.02). Patients with NTG had significantly (p<0.005) thinner CCT (506±55μm) than patients with POAG (567±53μm). As could be expected, OHT patients showed higher CCT values (592±41μm). CRF was significantly higher in the OHT (11.0±2.2) than in the POAG group (9.6±2.0) (p<0.006). For CH, we observed no difference between groups.

**Conclusion**

The ORA showed a good agreement with DCT and GAT for “normal” IOP, but we observed higher results compared to the other methods for high IOP values. We observed no correlation between CCT and IOP, but a weak positive correlation between CRF and CCT.
**433**

Influence of diabetes on central corneal thickness in type II ocular hypertensive patients

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**Purpose**
Are central corneal thickness values in ocular hypertensive patients comparable to that of non-diabetic patients? Is there some influence of diabetic disease on these values?

**Methods**
A sample of 271 patients has been distributed in 4 groups: 77 normal control patients (N), 74 ocular hypertensive (OHT), 59 diabetic patients without ocular hypertension (DM) and 61 ocular hypertensive type II diabetic patients (DMOHT).

Time from diagnosis and diabetes treatment are recorded. Also central corneal thickness (CCT), intraocular pressure (IOP) and diabetic retinopathy stage are also recorded.

SPSS 14.0 statistical program was used and one factor ANOVA, Krukal-Wallis and chi square tests, and Pearson calculation have been applied.

**Results**
Mean CCT in N group (536.50±38.14μm), and mean CCT in DM group (545.49±29.69μm) are not statistically different. Mean CCT in DMOHT group (570.45±33.59μm) and mean CCT for OHT group (570.51±31.26μm) are not statistically different.

Mean ECC difference between diabetic and non-diabetic subgroups is not statistically significant. However, the mean ECC difference between ocular hypertenive and ocular normaltensive subgroups is significantly high in both eyes (P < 0.000). Diabetes treatment has not influence on mean CCT intergroup comparisons. CCT and IOP correlate significantly in the whole sample for both eyes (P < 0.000). There was no linear correlation between mean CCT and age, nor between mean CCT and time of diagnosis.

**Conclusion**
Like OHT patients, DMOHT patients showed thick cornea. DM patients have normal CCT. Neither diabetes treatment, nor time of diagnosis are correlated to CCT. Corneal thickness is probably not related to the disease in DMOHT patients.

**434**

Correlation between global indices of short-wavelength automated perimeter and GDx VCC parameters

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**Purpose**
To correlate the main indices of short-wavelength automated perimeter (SWAP) with the peripapillary retinal nerve fiber layer thickness measured with the scanning laser polarimeter GDx VCC (Laser Diagnostic Technologies, Inc., San Diego, CA) in different diagnostic groups.

**Methods**
400 eyes were divided into 56 control eyes, 211 ocular hypertensive subjects, 67 glaucoma suspects and 66 glaucomatous patients, depending on intraocular pressure, optic nerve head morphology and standard automated perimeter (Humphrey 24-2 SITA standard).

Only one eye was randomly chosen for the study. SWAPs were performed with a Humphrey perimeter and the 24-2 full threshold algorithm. Pearson correlations between SWAP indices (mean deviation -MD- and pattern standard deviation –PSD–) and GDx VCC parameters (Nerve fiber indicator –NFL–, TNST average, superior average, inferior average and TNST standard deviation) were calculated for each group of subjects.

**Results**
Mild significant (p<0.05) correlations were observed between SWAP indices and most GDx parameters in the glaucoma suspect and the glaucomatosus groups. Few GDx parameters showed mild correlations with the indices of SWAP in the normal group, but no significant correlations were found in the ocular hypertensive group.

The TNST standard deviation exhibited the strongest correlation with the MD in the glaucoma group (r=0.33).

**Conclusion**
Laser polarimetry parameters showed mild correlations with SWAP defects in glaucoma subjects.

**435**

Diagnostic ability of GDx VCC to discriminate between healthy eyes, ocular hypertensive subjects and glaucoma patients

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**Purpose**
To evaluate the diagnostic ability of laser polarimeter GDx VCC to discriminate between healthy eyes, ocular hypertensive subjects and glaucoma patients.

**Methods**
417 eyes of 417 subjects were included. They were divided into 60 healthy controls, 289 ocular hypertensive subjects and 71 glaucoma patients. The GDx VCC parameters were compared among the study groups using analysis of variance. The receiver operating characteristic (ROC) curves were plotted for TNST average, superior average, inferior average, TNST standard deviation and nerve fiber indicator (NFI) between control and glaucoma eyes.

**Results**
All evaluated parameters of the GDx VCC showed significant differences between control and glaucomatous eyes and between ocular hypertensive eyes and glaucoma patients. NFI had the greatest area under the ROC curve (AUC=0.881). The AUCs for the inferior average, TNST standard deviation and TNST average were 0.834, 0.824 and 0.819, respectively. NFL presented a larger AUC than the other GDx VCC parameters.

**Conclusion**
The GDx VCC exhibited a good diagnostic ability to discriminate between healthy and glaucomatous eyes with damage in SAP. The best parameter was NFL.

**436**

Correlations between retinal nerve fiber layer measurements obtained with the GDx VCC and standard automated perimeter

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**Purpose**
To correlate the structural parameters of the retinal nerve fiber layer (RNFL) obtained by using the scanning laser polarimetry with variable corneal compensation (GDx-VCC) and the results of automated perimeter (SAP), in normal, ocular hypertensive, preperimetric glaucomas and glaucoma subjects.

**Methods**
423 eyes of 423 consecutive subjects were prospectively included in the study and classified depending on baseline intraocular pressure, optic nerve head appearance and SAP results into four groups: 87 normal eyes, 192 ocular hypertensive eyes, 70 preperimetric glaucomas and 74 glaucomatoses eyes. In the different diagnostic groups, Pearson’s correlations were calculated between mean deviation (MD), pattern standard deviation, and the fifty two threshold points of SAP and structural parameters of RNFL obtained with the GDx VCC. Regression’s curves for the strongest correlations were also calculated.

**Results**
Mild or no significant correlations were found in the normal, ocular hypertensive and preperimetric glaucoma groups. Nevertheless, the glaucoma group presented mild to moderate correlations between several GDx VCC parameters and the analyzed SAP variables. The strongest correlations were observed between the standard deviation TNST and the MD (r=0.460) and between superior average with the threshold point number 45 (r=0.496).

**Conclusion**
RNFL parameters measured with the GDx VCC presented mild to moderate correlations with the visual field indices and the threshold values of SAP in the glaucoma group.
**Poster session 3: Neuro-ophthalmology / Strabismology / Paediatric / History, Glaucoma, Physiology/Biochemistry/Pharmacology**

### # 437

**Evaluation of clinical and HRT II quantitative parameters to detect suspect and early glaucoma patients**

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**Purpose**
To compare the different clinical and HRT II quantitative parameters among patients divided in three groups: control, suspect glaucoma and early glaucoma.

**Methods**
The relative importance of each of a set of independent variables was assessed by stepwise discriminate analysis to identify and combine the most useful parameters of both clinical and HRT II methods. Afterwards the most significative original variables were combined to generate a new variable in such a way that the measureable differences between the groups are maximized by means of using a linear discriminate function (LDF) to generate a receiver operating characteristic (ROC) curve for each set of clinical or HRT II parameters. The differences between the ROC curves derived from LDF analysis by each method (clinical or HRT II) were tested for significance.

**Results**
Two functions were found: Clinical = 9.69 - 0.09*IOP + 0.05*A and a combination of O.C. = 9.69 - 0.09*IOP + 0.05*A for IOP and 0.757 ± 0.042 r for H, p<0.01.

**Conclusion**
Despite of good discriminate results for single parameters to detect suspect or early glaucoma patients, a linear combined parameters appeared more effective statistically than clinical and HRT II single ones.

### # 438

**Visualisation of reverse pupillary block using AC-OCT in primary pigment dispersion syndrome**

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**Purpose**
To describe morphologic characteristics associated with secondary IOP elevation due to pigment release after pupil dilation in primary melanin dispersion syndrome.

**Methods**
The history of a 49 year old male patient with primary pigment dispersion syndrome and secondary ocular hypertension is described. Besides detailed ophthalmologic examination including slit lamp biomicroscopy, gonioscopy as well as IOP and Laser-Flare measurement (KOWA FC-1000, Kowa, Tokyo, Japan) before and after pupil dilation, anterior segment optical coherence tomography was performed before and after Nd:YAG laser iridotomy and in medical mosaic.

**Results**
After pupil dilation IOP increased up to 46 mmHg at the right eye but no IOP elevation was recognised at the left eye. Measurement by Laser-Flare Cell Meter revealed no cells in undilated state but 13.4 cells/0.075 µl at the right and 4.0 cells/0.075 µl at the left eye after mydriasis. Nd:YAG laser iridotomy was recommended for the right eye to the patient recently. Iris condifguration detected by OCT demonstrates a concave shape before iridotomy more pronounced temporal than nasal which resolves after application of pilocarpin as well as after performance of Nd:YAG laser iridotomy.

**Conclusion**
The effect of Nd:YAG laser iridotomy as a prophylactic but potentially causal treatment in pigmentary glaucoma can be easily visualized by OCT as a fast and non-contact procedure.

### # 439

**Diagnostic coincidence of the technologies of analysis of image HRT, OCT and GDx-VCC**

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**Purpose**
To value the diagnostic coincidence and specificity between different structural parameters of the topography papular realized with the HRT3, the optic coherence tomography Stratus OCT 3000 and the laser polarimetry GDx-VCC.

**Methods**
There were included 41 eyes of 41 normal subjects (intraocular pressure <21 mmHg, normal optic nerve head morphology and standard automated perimeter), and 312 eyes of 312 consecutive patients sent by the ophthalmologist of zone for suspicion to suffer glaucoma. An analysis was realized to each of them by means of HRT, OCT and GDx-VCC and there was valued the diagnostic opposite coincidence. Considered to be criteria of damage a Moorfields Regression Analysis - MRA > 95% and a Glaucoma Probability Score - GPS > 0.51 to level of the HRT x NFL x 30 in the GDx and an average thickness or in a quadrant of the cap of retinal nerve fiber layer thickness with a value of p < 5% by OCT.

**Results**
The specificities of MRA and GPS were 85 % and 80%, in the GDx-VCC of 78%, and in the OCT of 71%. Between the suspects they found 113 positive cases by means of MRA, 153 by GPS, 101 by OCT and 64 by GDx-VCC.

**Conclusion**
It is necessary to possess the analyzers of image in the screening of the glaucoma since they contribute a high specificity, being major the contributed one for the HRT. The opposing specificity is not associated with a low number of positive cases and the diagnostic coincidence of the different analyzers is high Key words: glaucoma, diagnosis, HRT, OCT, GDx-VCC.

### # 440

**Influence of optic disc size in retinal nerve fiber analysis by OCT: could a customizable circular algorithm help to solve the problem?**

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**Purpose**
To investigate the correlation between two different type of optical coherence tomography circular scan algorithms for retinal nerve fiber layer (RNFL) analysis and optic nerve head (ONH) size in normal subjects.

**Methods**
94 eyes of 94 healthy subjects (60 females, 34 males) underwent ONH analysis performed by StratusOCT (Zeiss Meditec), with six radial scans centered on the optic disc (Fast Optic Disc scan). RNFL thickness was analyzed with two different type of circular scan: a 3.4 mm fixed-diameter ring (Stratus OCT, RNFL Thickness 3.4 scan) and a circular scan protocol which analysis a ring 2.27 mm far from a inner circle with a customizable diameter (Stratus OCT, RNFL Thickness 2.27’’ x disc scan). Correlation between RNFL thickness and optic disc size data was calculated for each algorithm.

**Results**
Analysis performed by 3.4 scan showed a significant (p<0.01) correlation with the optic disc area (OPD) (R = 0.47) and the vertical (VD) and horizontal (HD) disc diameters of the ONH (R = 0.51, ) and (R = 0.45). Analysis performed by 2.27’’ disc scan showed an value of 0.15, 0.21 and 0.17 for OPD, VD and HD respectively that are not statistically relevant (p = 0.05).

**Conclusion**
RNFL thickness measurements obtained by StratusOCT with 3.4 scan increase significantly with optic disc size, vertical and horizontal diameter. This relationship is not verified with 2.27’’ disc algorithm that seems to be less influenced by optic disc size. It is not clear if eyes with large ONHs show a thicker RNFL as a result of shorter distance between the circular scan and the optic disc edge or an increased amount of nerve fibers.
Prevalence of exfoliation syndrome in Pella-Greece

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Purpose To determine the prevalence of exfoliation syndrome (XFS) in the area of Pella, Greece.

Methods We studied a cluster, stratified sample of 708 individuals -45 years old (350 women and 358 men), representative of the population of Pella County. A comprehensive protocol recorded exfoliation findings before and after pupillary dilation, as well as signs commensurate with possible XFS. The intraocular pressure (IOP) was recorded in all cases.

Results Typical XFS was detected in 10% of the examined persons. The percentage of clinically evident XFS rose from 0% in the 45-54 age group, to 2% for the 55-64 age group, 23.1% for the 66-74 age group and 34% for the >75 group of patients. There was significant difference between the two sexes concerning the prevalence of XFS. Mean IOP was higher (20.04 mmHg) for the individuals with XFS versus those without (15.37 mmHg). Twelve patients with XFS (17.14%) had previously undiagnosed exfoliative glaucoma (XFG). Among the 70 persons with XFS, 60 (94.2%) had lens opacities, while 224 (35.5%) among the 630 persons without XFS had cataract.

Conclusion The prevalence of XFS in the area of Pella was determined to be 10% for individuals older than 45. There is strong correlation between XFS, glaucoma and cataract.

Peroxidase dismutase, glutathione reductase and glutathione peroxidase activities in the whole blood of exfoliation syndrome and exfoliation glaucoma patients

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Purpose Oxidative stress is implicated in the pathogenesis of both exfoliation syndrome (XFS) and exfoliative glaucoma (XFG). We aimed to study the whole blood levels of endogenous antioxidant enzymes, superoxide dismutase (SOD), glutathione reductase (GR) and glutathione peroxidase (GPX) in XFS and XFG patients.

Methods Deep frozen whole blood samples at -71 Celsius collected from 35 XFS patients including 11 XFG patients and 35 age- and gender matched controls, all of them operated on cataract, were analyzed for SOD, GR and GPX activity by using a commercial kit (Ransod, Random). For statistical analyses, Wilcoxon Signed Rank Test was used.

Results The whole blood enzyme activities in the SOD, GR and GPX in XFS/XFG groups were 1196±191 U/ml, 498.4±106.6 U/L, and 7281.5±1341.4 U/L, and in controls, 121.4±22.5 U/ml, 536.8±126.2 U/L, and 7689±18439 U/L, respectively. In the statistical evaluation, the respective p-values were 0.067, 0.169 and 0.263. In the subgroup of XFGs, apart from the XFS/XFG group, there were no statistical differences in the whole blood enzyme activities of SOD (p=0.394), GR (p=0.522) or GPX (p=0.905).

Conclusion The current study showed no significant differences in the whole blood enzyme activities in SOD, GR or GPX in XFS/XFG patients as compared to control group. This may implicate a proper function of these endogenous antioxidant enzymes despite of increased oxidative stress known to be involved in XFS and XFG.

The impact of clinical variables on the visual field damage of patients with pseudoexfoliative glaucoma

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Purpose To evaluate the relationship between the visual field global indices (Mean defect (MD), and Pattern Standard Deviation (PSD)) and different clinical characteristics in patients with pseudoexfoliative glaucoma (PEX).

Methods 56 consecutive patients with newly diagnosed PEX were included in this prospective cross-sectional study. Visual field was assessed using the 24-2 Swedish Interactive Threshold Algorithm (SITA). Clinical variables assessed were: Age, intraocular pressure (IOP), systolic and diastolic blood pressure, ocular perfusion pressure, central corneal thickness (CCT), and Doppler measurements in ophthalmic artery and posterior ciliary arteries. For each patient, the eye with the worse PSD of the visual field test was considered as the study eye. The relationship among clinical variables and visual field indices were analyzed by linear regression analysis.

Results The linear regression analyses showed that the age, IOP and CCT were correlated with both the MD (p=0.0001, p=0.0062, and p=0.0494, respectively) and the PSD (p=0.0013, p<0.0001, and p=0.0004, respectively).

Conclusion In patients with pseudoexfoliative glaucoma the visual field damage seems to be correlated with the age, IOP and CCT.

Commercial interest
# 445
Elevated levels of 3-Nitrotyrosine (3-NT) in human eyes with primary open-angle glaucoma (POAG)

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**Purpose** We have previously demonstrated the expression of inducible nitric oxide synthase in the trabecular meshwork from patients with POAG (EVER 2006, Abstract 2256). Therefore, we evaluated the involvement of peroxynitrite in the development of POAG by measuring the expression of 3-NT, a biomarker of oxidative damage.

**Methods** Aged-matched donor human eyes (3 controls and 4 with POAG) were fixed, embedded in paraffin and sectioned. Cellular location was determined by immunohistochemistry using a mouse anti-3-NT monoclonal antibody.

**Results** 3-NT immunoreactivity was observed in the anterior segment of POAG eyes: corneal pigmented epithelial and stroma cells of the ciliary processes, cells of the ciliary muscles, trabecular meshwork and Schlemm’s canal. In the retina, staining was observed in the ganglion, inner and outer nuclear cell layers. None of the donor control eyes showed any immunoreactivity for 3-NT.

**Conclusion** The presence of 3-NT immunoreactivity in human eyes with POAG suggests that peroxynitrite-mediated oxidative stress may have an important role in the pathophysiology of POAG.

# 446
Characterization of a rat model of glaucoma induced by laser

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**Purpose** To characterize a rat model on which glaucoma was induced by laser photocogulation of episcleral veins, trabeculum and limbal veins. The characterization of the model was based on the evaluation of the intraocular pressure (IOP), the retinal function and the number of functional retinal ganglion cells (RGCs).

**Methods** Male Sprague Dawley rats were used at 6 weeks of age. Three of the episcleral veins, the trabeculum and the limbal veins were photocogulated with a laser (532nm) in one eye: IOP (using a Tonolab) and electroretinogram (ERG) were recorded every 2 weeks during 11 weeks. Three months after photocogulation, RGCs were retrogradely labelled with a 5% Fluorogold solution applied to both superior colliculi. Seven days after labelling, retinas were flatmounted and were examined under a confocal fluorescence microscope equipped with a motorized stage. Functional RGCs were counted using an image analysis software (Image Pro Plus).

**Results** Treated eyes displayed a significant increase in IOP as soon as the first week after the procedure (32 mmHg versus 18 mm Hg in control eyes) and lasting for 7 weeks. The retinal function of treated eyes was altered since the ERG b-wave amplitude was significantly decreased when compared to control eyes (50% after 11 weeks). No differences were observed in the b-wave latencies. A reduction of 50% of the number of RGCs was observed in treated eyes when compared to control eyes.

**Conclusion** These results show that in the rat, eyes treated by laser photocogulation are a valuable model of glaucoma since they display typical characteristics, namely elevated IOP, followed by an alteration of the inner retinal function and retinal ganglion cell death.

# 447
Experimental glaucoma development in albino rabbits

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**Purpose** To compare four different glaucoma development models in albino rabbits, based on achieving a chronic elevation of intraocular pressure (IOP).

**Methods** A total of 16 animals were used for the glaucoma development model. Rabbits were divided in four groups. Caution of three vortex veins was performed in the first group (four animals) and cauter of four vortex veins in the second group (four animals). Three vortex veins were ligated in the third group (four animals) and ligation of four vortex veins was performed in the last group (four animals). IOP was measured by Tonovec rebound tonometer during six weeks follow up. The opposite unoperated eye served as control.

**Results** Cauterization of four vortex veins (second group) and ligation of four vortex veins (fourth group) achieved IOP elevation (40mmHg) but only for the first twenty four hours. The others groups achieved chronic elevation of IOP. Therefore, none of these methods developed chronic elevation of IOP.

**Conclusion** Vortex veins surgery in albino rabbits was not able to achieve chronic elevation of IOP. Therefore, none of this methods showed capacity to develop a glaucoma experimental model.

# 448
ATD perimetry in glaucoma and ocular hypertensive patients. A preliminar study

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**Purpose** The new ATD perimetry assesses contrast sensitivity thresholds for the three afferent sensory pathways of the visual system. Our aim is to compare contrast sensitivity to stimuli with different spatio-temporal frequencies in glaucomatous (G) and ocular hypertensive (OHT) subjects.

**Methods** Twenty three G and OHT subjects were selected from the ophthalmic clinic; all subjects signed an informed consent. Inclusion criteria were diagnosis of G or OHT, normal Farmworth Mn usel color test, ametropia less than 6 D, visual acuity over 20/30, absence of previous ocular surgery and experience in perimetry. Twenty locations and the foreau were evaluated in a 60° x 40° fovea-centered field. Eight gabor stimuli were used: 0.5cpd 12Hz, 0.5cpd-24Hz, 4cpd-2Hz and 4cpd 12Hz, modulated along the achromatic (A direction), 0.5cpd-12Hz and 4cpd-2Hz along the red/green (T) and blue/yellow (D) directions. Statistical analysis included ANOVA and Scheffé tests.

**Results** Mean thresholds for G and OHT were respectively, 0.4872 ± 0.5233 and 1.0077 ± 0.1922, for A. 0.5cpd-12Hz, 0.2926 ± 0.5925 and 0.2280 ± 0.2246 for stimulus A 0.5cpd-24Hz; 1.444 ± 0.5244 and 1.2671 ± 0.6138 for A 4cpd-2Hz; and 0.775 ± 0.1937 and 0.6851 ± 0.2579 for D 0.5cpd-12Hz. Significant statistical differences (p<0.05) were found between G and OHT groups only with these stimuli.

**Conclusion** These preliminary results show that certain stimuli may differentiate between G and OHT subjects. A larger population sample, including glaucoma suspects, is currently under study.

**Commercial interest**
**Poster session 3: Neuro-ophthalmology / Strabismology / Paediatric / History, Glaucoma, Physiology/Biochemistry/Pharmacology**

### 449

**FDT Matrix 24-2 threshold strategy for the evaluation of glaucoma suspects and ocular hypertensive eyes**

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**Purpose** To evaluate FDT Matrix 24-2 threshold strategy diagnostic performance in discriminating healthy subjects from glaucoma suspects (GS) and ocular hypertensives (OH) patients.

**Methods** 23 healthy subjects, 27 OH, 21 GS and 20 EG patients were recruited. Classification was based on optic disc and Humphrey Field Analyzer (HFA) perimetry criteria (glaucoma if PSD>95% and/or GHT outside normal limits). A complete ophthalmological evaluation, HFA (24-2, SITA standard) and FDT perimeter (24-2, ZEST strategy) were performed in all subjects. A severity score (from 1 for points with ≤5% probability up to 4 for probability ≤0.5%) was calculated from defective points on projection map and mean values (≤SD) in the 4 groups were compared at Mann-Whitney test. AUROC curves were created in order to assess MD, PSD and severity score diagnostic accuracy.

**Results** MD and PSD displayed chance discriminating power in OHs (AUROC=0.6) on both instruments. For GSs, FDT-MD and PSD have larger but not statistically significant AUROC than HFA. For EG, diagnostic performance of the two instruments is very similar with excellent AUROC (≥0.9). Severity score on the 2 instruments are similar for OHs, whereas significance limit was nearly reached (p=0.057) for GSs and significant difference appeared (p=0.002) for EG eyes with higher score on FDT.

**Conclusion** FDT Matrix 24-2 threshold strategy apparently does not offer advantages over HFA in OH and EG. In GS, statistically significant improvement in diagnostic accuracy was not demonstrated, probably because of insufficient sample size. Thus, the role of FDT Matrix in clinical practice needs further investigation.

### 450

**Epidemiology and follow-up of pigmentary glaucoma in Calabria**


**Eye clinic Magna Graecia University, Catanzaro**

**Purpose** To evaluate the incidence of pigmentary glaucoma (POAG) in Calabria.

**Methods** Subjects were patients newly diagnosed with pigmentary glaucoma during 2006 who came to our Centre of Glaucoma. We performed a full ophthalmological examination: Best Corrected Visual Acuity, slit lamp biomicroscopy, pachimetry, gonioscopy, measurement of intraocular pressure (IOP) with application tonometry/tomometric IOP curve, fundus oculi, Heidelberg Retina Tomograph, Heidelberg Retina Flowmeter, Nerve fiber analyzer, visual field, Optical Coherence Tomography, Ocular Blood Flow System, monitored ambulatory pressure, saccadic vessels doppler and transcranic doppler. Criteria for POAG were: pigment dispersion (mild, intermediate, severe), IOP greater than 21 mmHg, optic nerve damage and visual field loss.

**Results** A total of 87/150 (58%) patients presenting open angle glaucoma have been examined at the “Glaucoma Clinic Center” during the study (three times for year), 41/87 (47.12%) of them had POAG (open angle glaucoma 2-4); 22 females and 19 males (mean age 68 ± 12 years old, M±F). The male-female ratio was 1.1:1.

**Conclusion** The incidence of POAG in Calabria was 1:500 (0.2%). Owing the high frequency of POAG in Calabrian glaucoma patients we believe that performing at least a one time year follow up in these patients is helpful in preventing ocular complications.

### 451

**Use of Travaler® in routine clinical practice**

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**Purpose** To evaluate Travaler® in clinical practice.

**Methods** Travaler® is a computerized bottle holder that reminds patients to instill their medicine, assists in the administration of the drops, and records the dosing time for travoprost (Travatan®) or the travoprost/timolol fixed combination (DuoTrav®). ALCON, Inc., Fort Worth, TX. Technicians who dispensed the TDA completed a questionnaire regarding their experience with the device.

**Results** 3198 questionnaires were completed at 120 sites. Physicians alone usually decided to dispense the Travaler® (n = 1858, 58%) typically because patients forget to take their medication (n = 1613, 50%). The technician usually instructed the patient how to use Travaler® (n = 2774, 87%). Patients were typically very receptive to using the device (n = 2846, 64%) with their most common concern being monitored (n = 345, 11%). In the office the Travaler® typically required < 10 minutes to explain (n = 2855, 89%) and ≤2 minutes to initiate (n = 1873, 59%). Most offices reminded the patient to return Travaler® only at the visit in which it was dispensed (n = 1669, 54%) and most patients remembered to return it (n = 1613, 60%). The patients’ overall impression of Travaler® was favorable (n = 1846, 77%) and it typically took ≤5 minutes to discuss the findings with the patient (n = 1974, 63%). Most patients chose to continue using Travaler® (n = 1956, 82%).

**Conclusion** Travaler® offers a convenient method to monitor patient compliance with a high acceptability rate by the patient and clinical technicians.

### 452

**Local tolerability of Timolol 0.5% / Travoprost 0.004% (DuoTrav®) fixed combination in patients with open-angle glaucoma**

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**Purpose** To evaluate short/medium time tolerability (8 months follow-up) of timolol 0.5% / travoprost 0.004% (DuoTrav®) fixed combination in patients with open-angle glaucoma.

**Methods** Prospective, open trial in which 50 patients was involved with a 8 months follow-up. Inclusion criteria was primarily failure of beta-blockers or prostaglandin monotherapy and need for better IOP control and compliance. Exclusion criteria was patients with clinical contraindications to beta-blockers and history of allergy to prostaglandins. Follow-up parameters were: corneal erosions, conjunctival hyperemia, ocular symptomatology, intraocular pressure, break-up time, visual acuity. Primary objectives of the study was analysis of side effects and IOP shift. Secondary objective was patient compliance evaluated by questionnaire.

**Results** Patients compliance was improved despite to lower rate of side effects.No clinically relevant ocular signs or symptoms regarding lids, conjunctiva and anterior chamber were observed after the drug switch. DUOTRAV® produced a mean IOP reduction of 2.5 mmHg (range 1.8 to 3.3) (p<0.001). Visual acuity analysis showed no loss of letters at the end of follow-up and slit lamp examination showed a break-up time of 9.2 sec for monotherapy and 10 sec for fixed combination group (p<0.001).

**Conclusion** Results show that in daily dosing DUOTRAV® seems to be more effective respect to single drug treatment in reason of better tolerability, IOP control, compliance and clinic management.
**453**

Pharmacokinetics of preserved and preservative-free formulations of tafluprost are comparable in healthy volunteers

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**Purpose** Prostanoxin F2a (PT2a) analogues effectively lower intraocular pressure and are used as first-line treatment of glaucoma. Tafluprost is a newly synthesized PT2a derivative and the first PT2a analogue with a preservative-free formulation that may decrease the risk of side effects from chronic benzalkonium chloride exposure. This study investigated the pharmacokinetics (PK) of preserved and preservative-free tafluprost in healthy volunteers.

**Methods** A randomised, investigator-masked, single-centre, cross-over phase I study evaluated the PK and safety of preserved and preservative-free tafluprost 0.0015% eye drops. Both formulations were administered once daily for 8 days, with a wash-out period 4 weeks, to 16 healthy volunteers. Plasma concentrations, AUC0-last, Cmax and tmax were determined for AIP-172, the active metabolite of tafluprost. Adverse events and other safety parameters were analysed.

**Results** There were no statistically significant differences in PK between preserved and preservative-free formulations (mean SD AUC0-last 581.1±2799 vs 431.9±675.8 pg min/mL, P=0.462, Cmax 31.4±19.5 vs 26.6±18.0 pg/mL, P=0.294 respectively, median [range] tmax 10 [5-15] for both). Systemic bioavailability was comparable. Plasma concentrations of AIP-172 peaked at 10 mins and were low at all timepoints. Both formulations were cleared rapidly from the circulation. There were no unexpected safety findings. The incidence of ocular hyperaemia was similar with both formulations, being predominately of moderate severity with preserved tafluprost and mild severity with preservative-free tafluprost.

**Conclusion** Preservative-free tafluprost had similar PK to the preserved formulation and was generally well tolerated.

**Commercial interest**

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**454**

Reduction in intraocular pressure is equivalent between preserved and preservative-free formulations of tafluprost

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**Purpose** Tafluprost is a newly synthesized prostanoxin F2a (PT2a) derivative that reduces intraocular pressure (IOP) and is in development for the treatment of glaucoma. Tafluprost is the first PT2a analogue with a preservative-free formulation. This formulation may decrease the risk of side effects caused by chronic exposure to benzalkonium chloride. This study aimed to investigate the pharmacodynamics of preserved and preservative-free tafluprost.

**Methods** A randomised, investigator-masked, multi-centre, cross-over phase III study evaluated the pharmacodynamics and safety of preserved and preservative-free tafluprost 0.0015% eye drops administered for 4 weeks each in 43 patients with open-angle glaucoma or ocular hypertension. The primary variable was change from baseline in the overall diurnal IOP at 4 weeks. Adverse events and other safety parameters were also analysed.

**Results** A clear IOP lowering effect was observed with both formulations at Week 1. This effect was sustained and similar between groups at Week 4 with an estimated overall treatment difference (preservative-free – preserved) for the intent-to-treat population of 0.01 mmHg (95% CI, 0.46 to 0.49, P<0.09). There were no unexpected safety findings. Both formulations were well tolerated with most adverse events being ocular, and mild in severity. There were no serious adverse events or withdrawals due to adverse events in this study.

**Conclusion** Preservative-free tafluprost resulted in an equivalent IOP lowering effect compared with the preserved formulation, and was generally well tolerated.

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**455**

In vitro comparison of the oxidative and proapoptotic effects of preserved latanoprost, travoprost, bimatoprost and preservative-free tafluprost on conjunctival epithelial cells

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**Purpose** Prostanoxin F2a (PT2a) analogues are used for the treatment of glaucoma and ocular hypertension. This study compared the toxicity profiles of three commercially available preserved PT2a analogues—latanoprost, travoprost and bimatoprost—with tafluprost, a new preservative-free fluorinated PT2a analogue.

**Methods** Cells from an immortalised epithelial cell line from normal human conjunctiva (IOBA-NHC) were exposed (30 mins) to solutions of latanoprost, travoprost, bimatoprost (benzalkonium chloride [BAC] concentrations of 0.02%, 0.015%, 0.005%, respectively), Fg2a (0.0001%) and tafluprost. Membrane integrity/ cellular viability, pro-apoptotic effects and oxidative stress were assessed. Standard immunofluorescence was used to study morphologic patterns of cells under the same conditions.

**Results** Of the agents studied, preservative-free tafluprost resulted in significantly (P=0.001) higher membrane integrity (91.5 vs 37.4-latanoprost, 54.2-travoprost, 64.7-bimatoprost), lower oxidative stress (129.5 vs 339.7-latanoprost, 226.3-travoprost, 1810-bimatoprost) and lower proapoptotic effects (117.6 vs 412.3-latanoprost, 355.1-travoprost, 348.1-bimatoprost). Immunofluorescence showed that cell shrinkage increased in a BAC-concentration dependent manner, with the least shrinkage observed with BAC preservative-free tafluprost. The increase in harmful effects with ascending BAC concentrations of preserved PT2a analogues was observed for all parameters measured.

**Conclusion** These results suggest that tafluprost, a new preservative-free PT2a analogue, has low proapoptotic, proinflammatory and prooxidative effects in vitro.

**Commercial interest**

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**456**

Short-term effect of topical brimonidine tartrate on intrastrastromal pressure. An animal study

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**Purpose** To evaluate the effect of topical brimonidine tartrate on the intrastralional cornal pressure (ICP), due to it have been postulated that the brimonidine tartrate could have an adverse effect on postoperative flap adherence to the stromal bed after LASIK surgery, and that the endothelial pump plays an important role in flap adherence.

**Methods** Interventional, prospective study. The ICP was recorded for 45 minutes in eight eyes of rabbits treated with topical brimonidine tartrate three times daily for three consecutive days and in eight rabbit eyes treated with artificial tears (control group). All the measurements were performed by the same masked investigator.

**Results** The ICP averaged 4±2.9mmHg, 6.7±3.0mmHg and 9±4.5mmHg at 15, 30 and 45 minutes in the control group respectively. In the study group (brimonidine tartrate), the ICP readings were 2.7±2.9mmHg, 2.3±1.6mmHg and 8.5±5.5mmHg at the same time points, respectively. The differences in the ICP between both groups were statistically significantly different at 15 minutes (P<0.01), but neither at 30 nor 45 minutes (P=0.2 and P=0.8 respectively).

**Conclusion** The acute treatment with brimonidine tartrate produces significant changes in the ICP. This changes could help to explain the alteration in the flap adherence that have been reported.
Neuropeptide y protects retina neural cells from ecstasy-induced neurotoxicity

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Purpose The recreational drug 3,4-methylenedioxymethamphetamine (MDMA; ecstasy) causes neurotoxicity in the brain. Ecstasy consumers may experience visual effects, and significant levels of MDMA were detected in the human vitreous after lethal doses. It is also known that Neuropeptide Y (NPY) can be a neuroprotective agent. In this study, we investigated the putative neurotropic effect of MDMA in retina neural cells, and the potential neuroprotective effect of NPY.

Methods Rat retina neural cell cultures were prepared from newborn Wistar rats. The cells were exposed to MDMA (100-1600 μM) for 24 or 48 h at 37 °C or 40 °C. Cell viability was evaluated by MTT assay and propidium iodide staining. Retinal cells were identified by immunocytochemistry.

Results MDMA decreased retinal cell viability in a concentration-, time-, and temperature-dependent manner. The toxic effect of MDMA non-selectively affected different retinal cells (neurons and glial cells) and was dependent on caspase activation. In addition, the toxic effect was significantly reduced by ketanserin, a 5-HT2A antagonist, and by NPY, but the protective effect of NPY was more pronounced.

Conclusion These results show that MDMA is neurotoxic to retina neural cells, and the toxic effect can be exacerbated by hyperthermia. The toxicity of MDMA is partially mediated by the activation of the 5-HT2A receptor. Also, NPY is a potent neuroprotective agent against MDMA induced retina cell degeneration. These findings extend previous findings about the dangers of MDMA consumption. Further studies and a closer follow-up on the visual status of human consumers are evidently necessary.

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The suppression of early angiogenic markers by the antiangiogenic aptamer Macugen® dose dependent

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Purpose Pegaptanib sodium (Macugen®) is an RNA aptamer known to inhibit VEGF 165. We found it interesting to study the effect of Macugen® on the expression of the early angiogenic markers CEACAM1, PCNA and PINCH in a corneal alkali wound model where early events of VEGF induced neovascularization can be seen.

Methods 24 New Zealand rabbits were used. The animals received, in the right eye, subconjunctival injection of either 1.8 mg Macugen® (Mg), 0.45 mg Mg 0.2 mg triamcinolone (Kenalog®) or solute. The cornea was after the injection, with an alkali wound. The rabbits were killed after 48 hours, the corneas excised, fixed in 4% formaldehyde and embedded in paraffin. Sections were prepared and stained for morphology with eosin and immunohistochemically for PCNA and PINCH. Cells in the limbal stroma, positive for PCNA, were counted. RNA was isolated from paraffin sections from the limbal parts for RT-PCR analysis.

Results There was a significantly reduced number of PCNA positive cells in the Macugen® (Mg) 0.45 mg and triamcinolone sections compared to the solute group. In the Mg 0.2 mg sections there were a high expression of PCNA positive cells, with no difference compared to the solute group. RT-PCR showed expression of CEACAM1 in 6 out of 7 in solute group, 7 out of 7 in Mg 1.8 mg group and in 2 out 5 in Mg 0.45 mg group. There was no expression of CEACAM1 in the triamcinolone group. The results from PINCH staining will be presented.

Conclusion Pegaptanib in a low dose and triamcinolone inhibits the expression of the angiogenic cell adhesion molecule CEACAM1 and PCNA in alkali wounded rabbit corneas. These angiogenic markers may be useful for evaluating early events in angiogenesis.

Commercial interest

Comparison among bevacizumab, ranibizumab and pegaptanib on human umbilical vein endothelial cells viability, apoptosis and proliferation

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Purpose The aim of the present study was to compare the short term in vitro effects of anti-VEGF molecules available in ophthalmological practice in human umbilical vein endothelial cells (HUVECs).

Methods Cultures of HUVECs were established and treated during 24 h with different concentrations (including intravitreal concentrations used in intravitreal injections) of either bevacizumab, ranibizumab or pegaptanib diluted in culture medium. Controls were incubated with excipient solutions. Effects on cell viability (MTT assay), apoptosis (TUNEL assay), and proliferation (using BrdU) were investigated.

Results Viability of HUVECs incubated with bevacizumab, ranibizumab or pegaptanib did not significantly differ from controls in any of the concentrations examined. An increase in cell apoptosis by all anti-VEGF agents was found. In clinically used concentrations, apoptotic fold increase was higher in the bevacizumab group. A decrease in cell proliferation was found when HUVECs were incubated with any of the three agents.

Conclusion These findings indicate that all investigated molecules inhibit cellular proliferation without inducing cytotoxicity. Bevacizumab at concentrations identical to the dose normally used in intravitreal injections had a greater fold increase in apoptosis than the other anti-VEGF agents.

Histopathological findings after intravitreal injection of Linezolid

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Purpose To evaluate the retinal toxicity in rabbits of Linezolid after a intravitreal injection.

Methods We selected 10 New Zealand albino rabbits and divided them in two groups of 5. The rabbits in the treatment group received a 0.1 ml intravitreal injections of linezolid 2mg/ml, while the ones of the control group received 0.1 ml of saline solution. All the injections were performed in the right eye of the animals using 30-Gauge needle. We examine the fundus before and after the injection using indirect ophthalmoscopy to rule out pathologies or adverse effect due to the injection. 30 days after the inoculation of the drugs the fundus was reexamined and the eyes were enucleated. We carried out an anatomicopathological study of the retina to evaluate the effects of the drugs on it.

Results No sign of retinal toxicity were found in the fundus examination and in histological study of the retina of the two groups.

Conclusion Linezolid injected intravitreally appeared safe at concentration of 2mg/ml in albino rabbits. Intravitreal linezolid may be beneficial and safe and could be used in the treatment of bacterial endophthalmitis, particularly against resistant Staphylococcus aureus organisms.
**# 461**

**Photodynamic therapy with Verteporfin combined with intravitreal injection of Bevacizumab for central serous chorioretinopathy**

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**Purpose** To discuss the effect and outcome of a combined photodynamic therapy and intravitreal injection of Bevacizumab in treating central serous chorioretinopathy (CSC).

**Methods** A 52 year old patient with central serous chorioretinopathy (CSC) on his right eye was treated with intravitreal injection of Bevacizumab administered within 12 to 24 hours after standard PDT. Before, 1, and 3 month after treatment visual acuity and OCT examinations (retinal thickness) and fluorescein angiography were performed.

**Results** VA increased from baseline 20/40, to 20/20 after 1 month and remained 20/20 after 3 month. Subretinal fluid resolved completely after treatment. OCT- and FLA-findings are presented in our patient 1 and 3 month after combination therapy showed a normalized retinal thickness compared to baseline.

**Conclusion** Photodynamic therapy combined with injection of intravitreal Bevacizumab was very effective in our patient. Further experience is necessary to evaluate the treatment option of PDT and Avastin combination therapy esp. in chronic central serous chorioretinopathy (CSC).

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**# 462**

**Ocular and systemic distribution, and excretion of radioactivity following topical ocular administration of 14C-TG100801 to pigmented rabbits**

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**Purpose** To determine the ocular distribution, systemic exposure, and routes of excretion of single 0.84 mg/eye topical ocular doses of 14C-TG100801 to pigmented rabbits.

**Methods** Dutch Belted rabbits received single topical ocular doses of 14C-TG100801. One group received bilateral doses; urine and feces were analyzed by LSC. Two groups received unilateral doses and were analyzed by quantitative autoradiography.

**Results** There was considerable local absorption of 14C-TG100801 into ocular tissues and no appreciable systemic or contralateral eye exposure. Highest levels were in tissues of the anterior globe and radioactivity was translocated to the posterior globe (posterior sclera, choroid, and retina) for up to 96 hours. Little or no radioactivity was detected in the iris, ciliary body, lens, vitreous humor, or optic nerve head. In the whole body, radioactivity was primarily confined to the contents of the gastrointestinal tract. The primary route of elimination of was via feces.

**Conclusion** The results of this investigation demonstrate the efficiency of topical ocular instillation of TG100801 for treatment of local tissue while circumventing systemic exposure; there was considerable local ocular absorption of 14C-TG100801 into the anterior globe, progressing to the tissues of the posterior globe (posterior sclera, choroid, and retina). Radioactivity in the posterior globe was likely absorbed and translocated to this location because it is beyond the conjunctival fornix. There was no appreciable systemic or contralateral eye exposure and radioactivity was primarily excreted via the feces.

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**# 463**

**Intravitreal treatment with Erythropoietin (EPO) preserves visual function following ocular ischemia in rats**

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**Purpose** Erythropoietin (EPO) is a promising neuroprotective drug. It is known that EPO reduces apoptosis of retinal ganglion cells following axotomy or glaucoma in rats. Until now, functional aspects of this neuroprotective effect have not been addressed. We investigated effects of EPO on retinal and optic nerve function and on the survival of retinal ganglion cells following ocular ischemia.

**Methods** Ocular ischemia was induced by increase of the IOP to 120mmHg for 85 min in Brown-Norway rats. Animals were treated intravitreally with 4U/eye (n=12) during the time of ischemia, controls (n=16) received BSS instead. Visual pathway was investigated by VEP 4 days after ischemia. Potentials were evoked by frequency and luminance modulated flicker stimuli and recorded in awake freely-moving rats. Retinal function was evaluated by ERG 7 days after ischemia. Retinal ganglion cells were labelled retrogradely 4 days after ischemia and were quantified 6 days later in retinal flatmounts.

**Results** Both frequency and luminance modulated evoked potentials increased due to the application of EPO from 6±2% (mean in percent of the non-ischemic eye ± standard error) in control to 46±8% in treated animals and from 26±6% to 66±6% respectively.

**Conclusion** We found a sizable functional benefit of intravitreal injection of EPO following interruption of ocular blood supply. This suggests that administration of EPO is a viable therapeutic option in ischemic retinal diseases.

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**# 464**

**Relation of crystallin and ischemia/Reperfusion injury model of the rat retina**

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**Purpose** Ischemic damage of organs has hypoxic stress and reperfusion of the organs induces oxidative stress. Crystallin family is known as a protector of various kind of stress. We wanted to investigate crystallin expression and what kind of crystallin is associated to the ischemia/reperfusion injury (I/R) injury of retina.

**Methods** Twenty four Sprague-Dawley (SD) rats were used in this study. I/R injury was made by clamping optic nerve with central retina artery for 30 min and then set free. Groups were divided into normal control, 24h and 24h after I/R injury, 24h and 72h after I/R injury with pretreatment of Sn(IV) protoporphyrin IX dichloride (SnPP) and 24h, 48h and 72h after I/R injury with pretreatment of hemin. All of the vitreous bodies were obtained and two dimensional electrophoresis (2-DE) was performed.

**Results** Twenty-three spots were identified by MALDI-TOF/MS. Anti alpha A, alpha B, beta, phospho (ser19)-alpha B, phospho (ser15)-alpha B, phospho (ser39) alpha B-crystallin, extracellular signal-regulated kinases (ERK1/2), phospho-ERK1/2 and heme oxygenase-1 (HO-1) antibody were used for Western blotting. Beta-crystallins were increased 1.7 fold and 1.3 fold in the groups of I/R injury and I/R injury with SnPP pretreatment, respectively. Interestingly, I/R injured vitreous body showed the increased ERK1/2. However, I/R injured vitreous body pretreated with hemin for 24 h showed highly phosphorylated ERK1/2.

**Conclusion** This result might indicate that crystallin family is related to I/R injury. Phosphorylation of ERK1/2 was related with the hemin pretreatment.
**# 465**

**Methylprednisolone (MP) failed to protect retinal ganglion cells and visual function following ocular ischemia in rats**

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**Purpose** Methylprednisolone (MP) is commonly used to treat traumatic optic neuropathy and optic neuritis. It was neuroprotective in animal models of optic nerve crush, but showed negative effects after experimental autoimmune encephalitis. The present study investigated effects of MP on retinal ganglion cell (RGC) survival and on retinal and optic nerve function following ocular ischemia.

**Methods** Ocular ischemia was induced by increase in intracocular pressure to 120 mmHg for 55 min in adult Brown Norway rats. Animals were treated either with NaCl 0.5 mg/kg MP or 15 mg/kg MP intraperitoneally directly after and every 12 hours for 3 days. RGCs were labeled retrogradely 4 days after ischemia and were quantified 6 days later. Retinal function was accessed by scotopic and photopic electroretinogram (ERG) 7 days after ischemia. The investigation of the visual pathways by frequency and luminance modulated visual evoked potentials (VEP) was performed in awake freely moving animals on day four and ten after ischemia.

**Results** Ocular ischemia led to a decrease of labeled RGCs to 47±3% when obtained NaCl (means SEM), 46±3% with 0.5 mg/kg MP and 43±6% with 15 mg/kg MP ERG did neither differ significantly for scotopic nor photopic ERG potentials nor for the photopic 18 Hz flikker stimulation. Four days after ischemia the VEPs of rats receiving MP (0.5 and 15 mg/kg) were significantly higher compared to control. Ten days after ischemia potentials in both steroid groups decreased to control level.

**Conclusion** In our experiments treatment with MP did neither improve RGC survival nor retinal function. The delayed decrease of VEP indicated only a temporal benefit during steroids.

**# 466**

**Effects of (-)-bicuculline and gamma-aminobutyric acid on the Nic22 mediated stimulation of the ERG b-wave amplitude from the isolated superfused vertebrate retina**

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**Purpose** Nic22 (15 μM) stimulates the b-wave amplitude of vertebrate retina, up to 1.5-fold through its blocking of E/R-type voltage-gated Ca2+ channels. Assuming that these channels may trigger the release of the inhibitory neurotransmitter GABA, we tested the effect of (-)-bicuculline and GABA itself.

**Methods** We have used a superfused vertebrate retina assay, testing retina from bovine (Lüke et al., 2005: Brain Res Brain Res Protoc 16: 27-36). The retina was separated from the underlying pigment epithelium and mounted on a mesh occupying the center of the perfusing chamber. The electroretinogram was recorded in the surrounding nutrient medium via two silver/silver chloride electrodes on either side of the retina. The recording chamber containing a piece of retina was placed in an electrically and optically insulated air thermostat. The retina was dark-adapted and the electroretinogram was elicited at intervals of five min using a single white flash for stimulation.

**Results** (-)-Bicuculline increased the b-wave amplitude to a similar extent as observed in parallel recordings for low Nic22 (15 μM). The GABA effect was biphasic, and led to a transient augmentation after Nic22 application. Those retina segments which did not respond to Nic22 (15 μM), could also not be stimulated by bicuculline and vice versa.

**Conclusion** The stimulatory effect of Nic22 on the ERG b-wave amplitude is mediated by a Nic22-sensitive, probably Ca2+- voltage-gated Ca2+-channel triggered GABA release, and GABA itself may act on at least two different receptors.

**# 467**

**Arabinogalactan: a new ophthalmic vehicle for dry eye protection and treatment of corneal lesions**

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**Purpose** Dry eye syndrome and contact lens wearing may lead to desiccation of the corneal epithelium with abrasions and increased incidence of infectious diseases. Therefore, it is important a rapid regeneration when epithelium is compromised. Aim of the present investigation was to demonstrate that Arabinogalactan (AG), a natural polysaccharide from Larch tree, was well tolerated after ocular administration and exerted a corneal protective action.

**Methods** AG formulation was then subjected to specific investigations.

**Results** Rheological measurements highlighted a rheological non-viscous behavior of AG aqueous dispersions at concentration up to 10% with η value of 1.6 mPa.s. The rheological evaluation of mucin/hexehistive properties showed strong interactions with mucin, property useful to retain the formulation on the eye surface. 50% AG dispersion exerted a significant increase in healing rate of experimental corneal wound in rabbits since 27 h after the first treatment. The corneal epithelialization process produced a tissue stratification in the same features of native epithelium, restoring the normal multilayered architecture of the rabbit corneal epithelium. Finally, studies of cell proliferation on rabbit corneal epithelial cell culture were carried out. Solutions at AG concentrations from 5 x 10-3 to 1% enhanced the proliferation of the cells culture, showing cell viability values of 180% respect to the control after 48 h exposure time.

**Conclusion** These findings suggest that AG may be a new potential artificial tear for the prevention and the treatment of corneal wounds in dry eye syndrome and in contact lens induced abrasions since it does not interfere with vision because of its non-viscous feature.

**# 468**

**Ocular surface retention time and extensions of TFBUT of a lubricating eye drop**

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**Purpose** The tear film in subjects with ocular dryness is often unstable and incapable of maintaining the protective qualities that are intrinsic to its structure and function. Until recently, lubricating eye drops have provided only transient relief from ocular dryness. The purpose of these studies is to determine the protective properties of a lubricating eye drop, containing PG/PEG/HP-Guar, will extend ocular protection.

**Methods** To compare the ocular residence time of lubricating eye drops, subjects with dry, feeling eyes were administered a solution of Fluorescein isothiocyanate-dextran mixed with the eye drops. The residence time was measured by a scanning fluorometer. To compare TFBUT, subjects with moderately dry feeling eyes were administered a single drop of lubricating eye drop. After instillation, TFBUT was measured at 0, 5, 10, 15, 20, 30, 45, 60, and 90 minutes.

**Results** Tear film retention of the PG/PEG/HP-Guar product was significantly greater than a HPMC and a CMC lubricating eye drop. The product containing PG:PEG:HP-Guar was measured in the tear film as much as 37 minutes post instillation, while the HPMC and CMC drops were only 25 and 23 minutes, respectively. The PG:PEG:HP-Guar tear yielded a significantly greater increase in TFBUT from baseline at 45, 60, and 90 minutes post-instillation compared to eyes dosed with a CMC/Glycerin drop.

**Conclusion** Based on these results, it can be postulated that the extended duration of retention observed with the PG:PEG:HP-Guar tear allows the necessary time for restructuring of the unstable tear film into a more stable tear film. This leads to a long-term tear film stabilization and enhancement of TFBUT and ultimately, extended ocular surface protection.

**Commercial interest**
**# 469**

Nicardipine inhibits human conjunctival fibroblast proliferation, migration and attachment in vitro  

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**Purpose** To evaluate the effect of the calcium channel blocker nicardipine (NIC) on fibroblastic proliferation, migration, attachment and cell viability on human conjunctival fibroblasts in vitro

**Methods** Human conjunctival fibroblasts were incubated in a humidified incubator (37°C, 5% CO2), plus the doses of NIC to be tested. Fibroblastic proliferation at 18, 24 and 48 h was evaluated with a colorimetric assay based on the cleavage of the tetrazolium salt WST-1 by mitochondrial dehydrogenases in viable cells. Cell viability was evaluated at 4 h with the WST-1 assay and with trypan blue (TB) staining. Migration was determined in an artificial wound made in a confluent fibroblast monolayer. Cell free areas were monitored with digitalized images at 6, 18, 24 and 48 h. Fibroblastic attachment was evaluated at 24 h with the WST-1 assay. Groups 9 controls and 9 NIC concentrations (10-4, 7.5x10-5, 5x10-5, 2.5x10-5, 10-5, 7.5x10-6, 10-6, 10-7 and 10-8 M). Each experiment was carried out in triplicate and repeated 4 times.

**Results** NIC = 10-5 M showed cell viability = 98% with TB and =99% with WST-1 at 4 h. DEO = 100% at 10-4 M. NIC = 10-5 M at 18 h, and NIC = 7.5x10-6 M at 24 and 48 h showed significantly less proliferation than control groups. DEO at 7.5x10-5, 5x10-5, 10-5 M rank: NIC < 10-5 M showed significantly larger cell free areas at 18, 24 and 48 h. NIC = 7.5x10-6 M didn’t showed differences among the control groups. NIC = 7.5x10-6 M inhibited fibroblastic attachment in <25% at 24 h. DEO = 100% at 10-3 M.

**Conclusion** NIC have a significant inhibitory effect in vitro on conjunctival fibroblastic wound healing process with potential applicability in ophthalmic surgery.

**# 470**

Comparative study of commercially available antihistaminic drops in an animal model of early phase allergic conjunctivitis  

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**Purpose** To compare the relative efficacy of topical ocular antihistaminic drops with balanced saline solution (BSS) and benzalkonium chloride (BC) in the early phase of allergic conjunctivitis in animal model.

**Methods** 80 male guinea pigs were sensitized with intraperitoneal egg albumin (EA) and alum. Eighteen days after sensitization the animals were topicaly challenged by conjunctival instillation of EA. The animals were treated 15 minutes before and 15 minutes after challenge with commercially available drugs. Ketotifen with and without preservative, olopatadine, azelastine, spagumic acid and emedastine were tested. BSS and BC drops were tested as controls. Different drops were tested in the right and the left eye of each animal to avoid interanimal differences in allergic reactivity. The allergic reaction was measured by intravenous Evans blue extravasation (EVE) under general anaesthesia.

**Results** Two animals could not be used for evaluation. EBE was highest in the eyes treated by azelastine, olopatadine and preservative free ketotifen, and lowest in the eyes treated by spagumic acid. However, when the results of the right and left eyes of each animal were considered EBE was higher in the eyes treated by BSS and BC and lowest in the eyes treated by spagumic acid and emedastine.

**Conclusion** Spagumic acid and emedastine seem to be the most useful drugs to reduce EVE in an animal model of early phase allergic conjunctivitis.

**# 471**

Frozen fortified antibiotic in opthalmic solutions stability during 2 months  

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**Purpose** Fortified antibiotic ophthalmic solutions are regularly administered as an immediate treatment for bacterial keratitis. The fortified antibiotics were used to be self-prepared by nurses. To solve this problem, pharmacy staff studied the stability of three 5% solutions of vancomycin, amikacin and ceftazidim, prepared in aseptic conditions from parenteral antibiotic solutions.

**Methods** Solutions were frozen at -20°C. Portions of each solution were examined before storage and after a 75 days period. Cefazidim and amikacin were diluted to 0.9% sodium chloride and vancomycin in 5% dextrose. Over a 75 days period, physical, pharmaceutical (absorbance spectra) properties and sterility of each stock solution were studied.

**Results** The pH of amikacin (6.51), cefazidim (6.47) and vancomycin (3.77) remained stable during the 75 days period. Osmolalities also remained stable (367, 488 and 351 mOsm/L). There were no significant differences in the concentration, osmolality and pH of the three antibiotic solutions before storage and after 75 days of freezing. Over a 75 days period, stability of amikacin, cefazidim and vancomycin remained constant; no contamination was detected before storage and after 75 days.

**Conclusion** Topical fortified antibiotic solutions can be stored 75 days at -20°C. (15 days quarantine). After, these eye drops should be stored at 4°C and should be discarded after 3 days.

**# 472**

The pentadecapeptide BPC 157 antagonizes the effect of atropine in the rats’ eye  


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**Purpose** The goal of this study was to analyze the effect of the pentadecapeptide BPC 157 on miadriasis caused by atropine dropped into the rats’ eyes.

**Methods** 24 male Wistar Albino rats were used. All the experiments were performed according to the rules brought by the Local Ethical Committee. The animals were randomly assigned into two groups. The first received the two drops of pentadecapeptide BPC 157 (10μg/kg), and two drops of the atropine (25%). The other group received the two drops of atropine only. The animals were macroscopically assessed every hour.

**Results** The total miadriasis developed during 30-60 minutes after the application of atropine in both groups. The miadriasis reduction started 7 hours after the atropine application in animals treated with the pentadecapeptide BPC 157. On the other hand the reduction of the miadriasis in control animals started among 10-13 hours following the atropine application. The pentadecapeptide BPC 157 continuously accelerated the reduction of the miadriasis from hour to hour. The complete reduction to normal width of pupil was achieved 30 hours after the application of atropine in animals treated with the pentadecapeptide BPC 157, while in control animals the pupils width normalised 10 hours later.

**Conclusion** The pentadecapeptide BPC 157 has brought to earlier pupil width normalization after the single atropine application. It suggests the possibility of functional antagonism of the pentadecapeptide BPC 157 onto the atropine and its agonists.
**473**

**Characterization of COX-1 and -2 inhibition by nepafenac and its metabolite, amfenac**

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**Purpose** Nepafenac is a novel NSAID and a constituent of Nevarac ophthalmic suspension. We characterized the inhibition kinetics of prostaglandin H synthase-1 and -2 (COX-1 and -2) by nepafenac and its hydrolytic metabolite, amfenac.

**Methods** Purified rumin COX-1 and human COX-2 were reacted in 0.1 M KPi pH 7.2, 1 mM phenol, 1 µM heme and 0.075% Tween-20 at 30°C, monitored with an O2 electrode. Instantaneous (reversible) inhibition was assessed in enzyme-initiated reactions containing inhibitor and arachidonate (10 µM). Time-dependent inhibition was assessed in reactions initiated with arachidonate (100µM) after timed preincubation of enzyme with inhibitor.

**Results** Nepafenac and amfenac produced instantaneous (reversible) inhibition of both COX isoforms. Estimated Ki values were 2.3 µM (nepafenac) and 3.9 µM (amfenac) for COX-1 and 12 µM (nepafenac) and 26 µM (amfenac) for COX-2. The inhibitory potency of nepafenac did not increase when preincubated with either isomor, indicating that nepafenac is not a time-dependent inhibitor of COX-1 or -2. Amfenac exhibited time-dependent inhibition of both isoforms; the rate constants for the E1→E2 transition were 46 and 45 min-1 for COX-1 and -2, respectively.

**Conclusion** Nepafenac is a potent reversible inhibitor of COX-1 and -2. Amfenac also exhibits rapid, reversible inhibition, which progresses to time-dependent inhibition of both COX isoforms. This suggests that nepafenac, which rapidly accumulates in the aqueous humor after topical application, contributes to the initial suppression of ocular inflammation and prostaglandin formation, whereas amfenac generated by tissue hydrolases accounts for the sustained suppression of prostaglandin formation.

**Commercial interest**
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**Course 1: Amniotic membrane in ophthalmology: Applications and limitations**

### 1311

**Amniotic Membrane Transplantation (AMT): Historical overview**

**DILA HS, NOTTINGHAM**  
**Division of Ophthalmology, University of Nottingham, Nottingham**

**Purpose** To provide an overview of the history of AMT in ophthalmology

**Methods** Historically, a child born with the fetal membranes intact (caul), was considered to be lucky. Early Scottish sailors born with unruptured membranes carried a dry remnant of the caul, ‘the fairy how’ to protect them from the sea. With the healing qualities of the Amniotic membrane (AM) becoming substantiated by scientific research, this folklore of bygone days is rapidly becoming a clinical reality

**Results** Davies (1910) was the first to advocate the therapeutic use of AM in skin transplantation. Following this, several reports of its use in burns and other surgical procedures appeared. The use of AM in ocular surgery was first suggested by de Roth and Soniby in the management of chemical burns of the eye. Thereafter, for no evident reason, its use was abandoned or went unreported until recently. The early 1990s can be taken as a suitable starting point in tracing the modern history of the use of amniotic membranes in ophthalmic surgery. In May 1992, JF Battle and FJ Perdomo presented a paper entitled ‘Placental membranes as a conjunctival substitute’ to the annual congress of the Dominican Ophthalmology Society (Puerto Plata). Battle traced the use of “Soviet tissues” as allografts in conjunctival, tarsal, orbital, and tendon surgery. He undertook a study and determined the true nature of the “Soviet tissues” to be placental membranes. In June 1992 the work was presented at the Bascom Palmer Alumni Meeting in Miami, Florida, and later as a scientific poster (925) at the AAO Annual Meeting (Chicago Nov. 1993)

**Conclusion** From these on, SC Tseng, from Bascom Palmer, Miami, together with innumerable colleagues further advanced and developed this concept of the use of amniotic membrane in ophthalmic surgery

### 1312

**Clinical applications and benefits of amniotic membrane**

**MAHARAJA S, NOTTINGHAM**

**ABSTRACT NOT PROVIDED**

### 1313

**Amniotic Membrane Transplant (AMT): Evaluation of Outcomes**

**DILA HS, HOPKINSON A, NOTTINGHAM**  
**Division of Ophthalmology, University of Nottingham, Nottingham**

**Purpose** To describe the importance and methods of evaluating outcomes of AMT.

**Methods** Amniotic membrane transplantation has become an established technique in the management of a wide range of ocular surface disorders. It is used as an adjunct in a variety of ocular surface, glaucoma and oculeo-plastic surgical procedures. There are two major limitations to the use of the membrane: 1) Poor standardisation of the membrane due intra and inter donor variations and differences induced by processing procedures. 2) Lack of definitive outcome measures to evaluate outcome. In this context we have developed a system based on ‘Purpose’ of the membrane whether intended as a patch or a graft and ‘Objective’ of its application. 1) to establish epithelial cover in an area where none existed; (ii) to prevent corneal perforation in eyes at risk because of stromal melting; (iii) to limit scarring where the clinical likelihood was high or where scarring (symblepharon/adhesions) previously existed; and (iv) to limit inflammation and neovascularization. Based on the above factors, outcomes are described as Success (when both purpose and objective are achieved); Partial success (when purpose and/or objective are not fully achieved) and failure (when the objective is not achieved).

**Results** Using this measure, we found that in an evaluation of 74 procedures, success was achieved in 22% to 62% depending on the indication. Lack of proper grading of extent or severity of the underlying condition also determines outcome and should be accurately undertaken to ensure proper evaluation.

**Conclusion** By adopting defined criteria such as described above a true understanding of the efficacy of the membrane for different conditions can be obtained and a valid comparison with existing other procedures can be made.

### 1314

**Limitations of the amniotic membrane**

**HOPKINSON A, NOTTINGHAM**  
**Division of Ophthalmology and Visual Sciences, Nottingham**

**Purpose** The Amniotic Membrane (AM) has become a useful adjunct in the management of many ocular conditions. Several mechanisms of action have been attributed to the beneficial effects of AM based on its structural and biochemical composition, which has lead to vast reports in literature. However, as well as these studies often lacking proper and adequate controls, not all the reported mechanisms are scientifically substantiated. Very little is known of the inter and intra-donor variability within the AM. Location in relation to the placenta, duration of pregnancy, parity, gravidity, onset of labour and even age and race of the donor are all potential sources of biological variation. In addition, the effects of ‘Handling’ (collection, processing, preservation and post preservation processing) on the AM are not fully elucidated, and the subsequent clinical implications have not been previously considered.

**Methods** To assess biological variation, fresh AMs were analysed for a range of molecules at the gene and protein level by polymerase chain reaction (PCR), and by immunohistochemistry, western blotting and 2-D electrophoresis, respectively. Subsequently, the effects of handling on comparable membranes were assessed at the protein level, and by electron microscopy.

**Results** Inter and intra-donor variations in the biochemical composition and function of the AM were demonstrated. Furthermore, non-standardised handling procedures result in a devitalised AM with a substantially altered protein composition further compounding AM variability.

**Conclusion** There is a degree of inter and intra-AM variation, which is further complicated by handling. Therefore, standardisation of the membrane supplied for widespread clinical use is an important challenge that lies ahead. Without this, clinical outcomes may vary.
Future Directions: Standardised membranes and synthetic membranes

GICQUEL JJ (1, 2)
(1) Ophthalmology / Jean Bernard University Hospital, Poitiers
(2) Division of Ophthalmology and Visual Science, Nottingham

The amniotic membrane (AM) has been used for almost a century in reconstructive surgery of various parts of the human body, including the oral cavity, bladder, and vagina. More recently, it has found application for treating ocular burns and ocular surface disorders, including conjunctival and corneal defects. One placenta can provide material sufficient for more than 20 surgeries. Limbal epithelial cells are also cultured and expanded on denuded AM. Until now there have been very few studies on the proteins present in AM and responsible for its anti-inflammatory, anti-scarring and anti-angiogenic properties. A better knowledge of the biochemical composition and function of the AM will help develop standardised AM, both for culture and surgery as well as later on synthetic membranes. Thru a review of published studies and of the research performed at the Division of Ophthalmology and Visual Sciences we will focus on the necessity of using standardised membranes for both limbal epithelial cells culture and surgery. We will also show how in the future we expect to build a bio-engineered or even a fully synthetic AM.
**Course 2: Corneal infectious diseases**

* 1321
Bacterial keratitis

BOURCIER T
Ophthalmology, Strasbourg

Bacterial infection of the cornea is a sight-threatening process requiring a prompt management. This course will focus through many clinical cases, on diagnosis and therapeutic advances.

* 1322
Parasitic infection

KODIRIAN L
Lyon

ABSTRACT NOT PROVIDED

* 1323
Corneal fungal infections: practical diagnosis and treatment

GICQUEL J [1, 2]
(1) Ophthalmology / Jean Bernard University Hospital, Poitiers
(2) Division of Ophthalmology and Visual Sciences, Nottingham

**Purpose**
To describe key aspects of fungal infections of the cornea and give guidelines for modern diagnosis and treatment.

**Methods**
Thru a review of published studies and personal observations we will apprehend physiopathology and treatment of corneal fungal infections.

**Results**
Trauma is the most important predisposing cause. Prior application of corticosteroids is also an important risk factor. It has also recently been discovered that a specific contact lens solution had increased the risk of corneal fungal infections. In many cases it is difficult to differentiate bacterial keratitis from fungal keratitis on clinical judgment alone. Culture remains the cornerstone of diagnosis. Direct microscopic examination of fungal structures in corneal scrapes and biopsies permits a rapid presumptive diagnosis. Various new antifungals have been evaluated recently and show promising results. However, sometimes medical therapy may fail and surgery is necessary.

**Conclusion**
Fungal infections of the cornea are more than ever an important cause of ocular morbidity. A proper understanding of agent and host factors involved in these infections is indispensable.
Course 3: Pediatric ophthalmology examination

* 1331 Measuring vision in children

MARTIN L
Clinical Neuroscience, Karolinska Institute, Stockholm

Purpose This lecture will describe and discuss techniques for examining vision in children, including rules of thumb for judging visual behaviour in newborns and toddlers.

Results For some examination techniques (e.g. for visual acuity and perimetry), normal age-corrected values are established, at least for elderly children, but most apparatus and computerized methods are customized for adults, and normal values for children are not available. This is sometimes due to the widespread misunderstanding that preschool children are unable to cope with e.g. computerized perimetry. Therefore we often have to create normal values ourselves. In many cases it’s better to get some information about a child’s vision using non-conventional methods, than getting no information using well-established, evaluated methods.

* 1332 Examining children with multiple handicaps

ARING E
Gothenburg

Purpose This lecture will focus on tricks and tips for the clinical examination as well as methods suitable for examining children with multiple handicaps. Ophthalmologic findings in different handicaps will also be discussed.

Results Strabismus, refraction abnormalities, and amblyopia are common in children with multiple handicaps, as a consequence this group of children often need frequent eye examinations. For normal children many tests are available and values are well established. To make the testing of children with multiple handicaps as trouble-free as possible, the investigator has to know how to handle this group of children, and what methods to choose. In addition, to get any information at all, non-conventional methods are to be used some times.

* 1333 Measuring and evaluating binocular vision

BRALITAS E
Unit of Ophthalmology, Karolinska Institute, Stockholm

The development of the accommodation and vergence systems and their mutual interaction play an important role in the development of normal binocular vision. This lecture will focus on testing procedures used to evaluate accommodation and vergence functions in children. Emphasis will be made on tests that can detect accommodative and vergence disorders and tests that can distinguish a vergence disorder from an accommodative disorder. The talk will also address the normal and abnormal development in binocular vision in children and how testing procedures should be adapted to children’s age and level of binocular vision.

* 1334 Assessing eye movements

BERK T
Lumir

Assessment of various aspects of visual function and eye movements in infants and children will be discussed. Abnormal eye movements in children may result from abnormal visual development or they may be a result of underlying neurologic and/or ophthalmologic disease. Eye movements in healthy infants will be described (OKN, vergence, saccades). Course provides a practical guide to the examination and clarification of eye movements and includes some examples of ocular motility problems (e.g. horizontal gaze palsy, Duane retraction syndrome, infantile esotropia, cranial nerve palsy...)

EVER 2007 - Abstract book

77
Course 4: Introduction in statistics

* 1341
Introduction in statistics I
MOJON D
St. Gallen
ABSTRACT NOT PROVIDED

* 1342
Introduction in statistics II
MOJON - AZZIS
St. Gallen
ABSTRACT NOT PROVIDED
* 1351
Endophthalmitis epidemiology - types of bacteria involved, their frequency, settings where it occurs (lens types etc)
KOBIKIAN L
Lyon
ABSTRACT NOT PROVIDED

* 1352
Preventative measures - wound design in cataract surgery, wound integrity and the use of intracameral antibiotics
MONTAN P
Stockholm
ABSTRACT NOT PROVIDED

* 1353
Diagnosis - when to suspect, what to do, how and when, what to expect from the microbiology lab.
The role of PCR in the diagnosis
BODAGHI B
Paris
ABSTRACT NOT PROVIDED

* 1354
Choice of antibiotics and how long to treat
COCHEREALIC
Ophthalmology, Angers

**Purpose** In bacterial endophthalmitis, antibiotics are the main treatment. They must be started as soon as possible after the diagnosis is suspected. Intravitreal antibiotics are mandatory since the vitreous is the key compartment, topical antibiotics are optional and systemic antibiotics are still controversial.

**Methods** Initially, a combination of two bactericidal antibiotics are required for a synergistic and broad spectrum antibacterial coverage since the causative organism is not known when the antibiotherapy is started. Gram-positive organisms (especially coagulase-negative) are the most frequent (90%), gram-negative organisms (10%) less frequent cause very severe endophthalmitis. For intravitreal injection, the combination of vancomycin plus amikacin or ceftazidim are the most frequently used, due to their efficiency on gram-positive organisms. In case of further deterioration due to a gram-negative organism, a switch to ceftazidime-amikacin or ceftazidime-tobramycin might be useful. One or two intravitreal injections performed within the first week are usually sufficient.

**Results** Fortified topical antibiotics are indicated only to treat an associated superficial abscess, but they do not kill intravitreal organisms.商ially available topical antibiotics are prescribed to decontaminate the ocular surface Systemic therapy is controversial. If used, a 5 to 8 day combination of antibiotics with favourable intraocular kinetics like intravenous imipenem and a new fluoroquinolone can be used.

**Conclusion** A prompt, wide spectrum and bactericidal initial intravitreal antibiotherapy is the key initial treatment in bacterial endophthalmitis. Its association with other routes of administration will depend on the severity of the infection, nature of the organism, and response to therapy.
Course 6: Ophthalmologic pathology: Basics, updates and new insights

NO ABSTRACTS FOR THIS COURSE

Course 7: European Board of Ophthalmology (EBO) exam course

NO ABSTRACTS FOR THIS COURSE
Oral Presentations

THURSDAY
• Sessions on Thursday ........................................... 82-122

FRIDAY
• Sessions on Friday ........................................... 123-163

SATURDAY
• Sessions on Saturday ........................................... 164-203
* **2111**
Lyme disease (Borreliosis)

BOURCIER T
Strasbourg

ABSTRACT NOT PROVIDED

* **2112**
Bartonellosis

GARWEG IG
Swiss Eye Institute, Bern

**Purpose** Bartonellosis, a rarely diagnosed bacterial zoonosis caused by Bartonella species is capable of inducing a number of neurological diseases, such as nervous paresis and Parinaud syndrome, and it may affect the eye, and namely the retina.

**Methods** Cats are the main reservoir for human-pathogen species, i.e. Bartonella henselae, the etiologic agent in cat scratch disease, but dogs can also be infected with Bartonella parasites. Ocular bartonellosis is widely associated with Bartonella henselae, is most often associated with ocular complications, which may include Parinaud ocuularpapillitis, neuroretinitis, and focal retinochoroiditis.

**Results** The typical clinical presentation of neuroretinitis and Parinaud syndrome will be presented and personal treatment experience will serve the basis for discussion. Numerous other clinical patterns have been reported occasionally. The diagnosis of ocular bartonellosis relies on the identification of suggestive clinical signs in conjunction which is confirmed by serological and eventually aqueous humour testing. B. henselae-associated ocular disease is usually self-limited but go along with transient more or less significant visual complaints. If the infection is clinically severe or sight-threatening, treatment may be considered, usually with doxycycline or erythromycin, eventually combined with rifampin.

**Conclusion** B. henselae infection may be significantly more common than clinically diagnosed and should be considered in patients with Parinaud ocuularpapillitis syndrome, neuroretinitis, or focal retinochoroiditis, particularly when there is a history of pet or flea exposure.

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* **2113**
Toxocariasis

HERBORT C
Lausanne

ABSTRACT NOT PROVIDED

* **2114**
Leptospirosis

LIESENFELD O
Berlin

ABSTRACT NOT PROVIDED
Brucellosis

DE SMET M (1, 2)
(1) Division of Vitreo-retinal and Uveitis, Department of Ophthalmology, ZNA Middelheim, Antwerp
(2) Department of Ophthalmology, Academic Medical Center, University of Amsterdam, Amsterdam

ABSTRACT NOT PROVIDED

Ocular toxoplasmosis due to Toxoplasma gondii is presumably the most frequent infectious cause of posterior uveitis throughout the world. Ocular toxoplasmosis is characterized by recurrent episodes of necrotizing retinochoroiditis thought to be caused by both proliferation of live organisms that emerge from tissue cysts within the retina and an associated inflammatory reaction. Toxoplasmocrinetochoroiditis can be associated with severe morbidity if disease extends to structures critical for vision, including the macula and optic disk, if there is damage to the eye from inflammation, or if there are complications such as retinal detachment or neovascularization. Clinical diagnosis is helped by ancillary laboratory testing, Witmer-Denmon's coefficient and western blot. The current approach to treatment of severe toxoplasmatic retinochoroiditis involves finite courses of anti-Toxoplasma gondii drugs, with or without corticosteroids, in emergency. New strategies for treatment of ocular toxoplasmosis are tested and will be developed during present SIS.

Prion disease

BODAGHI B
Ophthalmology, Paris

Prions are a new form of pathogens made by proteins and lacking genetic information such as DNA or RNA sequences. These proteins may transmit certain phenotypes that are inherited in a non-Mendelian manner. Prions are possibly the primitive form of life, existing before or with self-replicating RNA. The abnormal form of prion proteins results in a misfolded molecule, becoming resistant to different major neutralizing agents. Prion protein (PrP) is expressed in many tissues and is required for susceptibility to scrapie and other prion diseases. Creutzfeldt-Jakob disease (CJD), the most common form of prion diseases, is a rare and fatal neurodegenerative disorder with a worldwide incidence of 1-1.5 per million. Different animal models have been developed in order to study the pathophysiology and transmission of prions. The presence of the pathologic isoform in the retina seems to be correlated with disease progression during the predinal and clinical stages, perhaps using the inner plexiform layer as a first entry site and diffusing from the brain using a centrifugal model. On the other hand, prions may spread within the CNS via central axons of the visual pathways following intraocular inoculation. All these considerations are important for the better understanding of the disease and its variants in humans.
### 2121

**Age-related cataracts in a German population-based study (KORA S4)**

**GRAF F (1), KLOPPZ (2), ILLEG T (2), GIEGERT C (2), WELZL G (1), LOWEL H (2), ZAHLMANN G (2), HOLLE R (4), WIEHMANNHE (2)**

(1) GSF-National Research Center for Environment and Health, Institute of Developmental Genetics, Neuherberg.
(2) GSF-National Research Center for Environment and Health, Institute of Epidemiology, Neuherberg.
(3) Siemens AG, Medical Solutions, Erlangen.
(4) GSF-National Research Center for Environment and Health, Institute of Health Economics and Health Care Management, Neuherberg.

**Purpose**
The population based study in the region of Augsburg (Germany), KORA, is used to evaluate cataract prevalence.

**Methods**
2,786 randomly selected probands were asked in a standardized interview for the presence of cataracts. A set of SNPs of functional candidate genes was tested for association.

**Results**
115 probands reported cataracts (4.1%), of them suffered also from glaucoma and are excluded from the further analysis. The prevalence increased from 0.9% (<25 years) to 4.9% (55-64 years) and 12.6% (65-74 years); there was no sex effect observed. After adjustment for age, there was no significant association between cataract and blood pressure, diabetes, smoking, body mass index and alcohol consumption. Additionally, we investigated the hypothesis that the age-related formation of cataracts might be associated with SNPs in genes, which have been shown previously to be involved in hereditary cataracts. Among 14 SNPs tested, one polymorphic site in the CRIEG4 gene showed significantly negative association with cataracts (OMD ratio 1.97, p = 0.013).

**Conclusion**
Our data set could not confirm the major risk factors for age-related cataracts, which might be due to the small numbers of cataracts. Nevertheless, even in the small sample size, an association to a well-known cataract-causing gene has been observed, which needs to be confirmed in independent studies.

### 2123

**Genotype–phenotype correlation in AMD: The influence of complement factor H polymorphism**

**MANTEL J (1), DROOZ J (1), AMBRESI N (1), SCHRODER T (2), MUNIER F (1)**

(1) University Eye Hospital Jules Gonin, Medical Retina, Lausanne.
(2) Institut de Recherche en Ophtalmologie, Sion.

**Purpose**
Complement factor H (CFH) Y402H SNP rs1061170 shows strong association with AMD. Phenotypic concordance of AMD is known from sibling and twin studies. Studies about genotype-phenotype associations showed inconsistent results. This study investigates the association of CFH Y402H SNP with AMD in a Swiss population, and the genotype-phenotype association, particularly in early stages of AMD.

**Methods**
A total of 473 AMD patients and 69 control individuals were enrolled. Colour fundus photographs were graded according to the International Classification and Grading System for AMD. The SNP rs1061170 was evaluated for each patient. Statistical analysis was restricted to individuals with complete clinical data (420 AMD cases and 50 controls).

**Results**
An OR of 3.05 was found for the presence of at least one risk C-allele, and OR 9.3 for the homozygotic genotype. Individuals over the mean age of 76y and homozygous for the risk allele C showed more frequently late forms of AMD. No phenotypic association was found for drusen size, drusen covered surface, drusen localization nasal to the disc, and pigmentary changes. A trend for central drusen location was found in patients homozygous for the risk allele C. [p = 0.051]. Peripherial drusen were significantly more frequent in individuals with the risk allele C, particularly if homozygous [p = 0.027].

**Conclusion**
This study confirms the risk association of the CFH Y402H polymorphism with AMD for the Swiss population. A genotype-phenotype association could be found for early forms of AMD, however only for some drusen features. Additional genetic factors are likely to influence the drusen phenotype. Further careful research is needed to better understand the genetic influence on AMD-phenotypes.

### 2124

**The ocular and systemic factors associated with diabetes mellitus in the Beijing eye study**

**LIBOND T (1, 2), XH J (3), XE X (3), WANG T (3), JONAS J (2, 3)**

(1) Dept. of Ophthalmol., II University, Naples.
(2) Dept. of Ophthalmol., Medical Faculty Mannheim, Univ. of Heidelberg, Mannheim.

**Purpose**
To determine the ocular and systemic factors which are associated with diabetes mellitus in the adult population in rural and urban China.

**Methods**
The Beijing Eye Study, a population-based, cross-sectional cohort study was repeated in 2006, with 3251 aged 40+ years subjects participating. Blood samples were available for 2773 (85.3%) subjects. All participants underwent an ophthalmic examination, anthropometric measurements and blood biochemical analyses. Diabetes mellitus was defined by a fasting plasma glucose concentration ≥7.0 mmol/l or by a self-reported history diagnosis of diabetes.

**Results**
By the criteria above defined, 314 subjects were diabetic (prevalence rate: 12.0%). In binary regression analysis, presence of diabetes mellitus was significantly associated with higher age, body mass index, systolic blood pressure, triglyceride concentrations, and intraocular pressure, and lower high-density lipoprotein level and diastolic blood pressure. Diabetes mellitus was not statistically associated with the degree of cortical and or subcapsular cataract, size of the optic disc, neuroretinal rim and alpha and beta zone of peripapillary atrophy, retinal vessels diameters or abnormalities, retinal microvascular abnormalities, refractive error, and prevalence of glaucoma and early or late stage of age-related macular degeneration.

**Conclusion**
In this population-based setting, diabetes mellitus is not associated with optic disc, rim and peripapillary atrophy measurements, retinal vessel diameters and microvascular abnormalities, and prevalence of age-related macular degeneration. Although diabetes mellitus is significantly correlated with higher intraocular pressure, it is not associated with glaucoma.
2125

Congenital aniridia

POPPER M, BAUSZ M, TURI E, SENYI K, KNEZY K, SUVEGES I

Ophthalmology, Semmelweis University, Budapest

Purpose Our purpose was to analyze the various ocular manifestations, treatment options, and the possible outcome of congenital aniridia (CA) a rare and severe developmental disorder.

Methods 52 eyes of 26 patients were regularly checked with full eye examination including anterior segment photography. Electrophysiology, OCT, UBM, and glaucoma testing was done if appropriate. The mean age at the last examination was 17.6 years (range: 2 months – 72 years).

Results The best corrected visual acuity was 0.2 (ranging from no light perception to 0.6). 20 cases were sporadic and 6 cases showed autosomal dominant inheritance in 3 families. A deletion including band p13 of the short arm of chromosome 11 was found in one familial and one sporadic case. Associated ocular signs were nystagmus (88.5%), congenital cataract (61.5%), and ectopia lentis (33.8%). 14 eyes of 16 cases needed cataract surgery. Secondary glaucoma occurred in 24 eyes of 12 patients, with severe outcome in 15 eyes of 8 patients despite treatment. Aniridic keratopathy occurred in 23 eyes of 12 patients, in 3 cases keratoplasty was performed. One patient had aniridia on one eye, and iris coloboma on the fellow eye confirming the common origin of the two diseases. Interestingly, no Wilms tumor occurred in our series, but mental deficits were found in 4 patients.

Conclusion Secondary glaucoma is the most severe complication of CA. It can appear in relatively young age, is relatively resistant to treatment, and the earlier the onset, the worse the prognosis. Risk factors for vision loss included eye surgeries at young age. Moreover, the outcome of cataract surgery is limited by foveal hypoplasia and the progression of glaucoma.

2127 / 245

Family with Brittle Cornea Syndrome

TOSOLINDIS IH (1), MCKEAG D (2), AHU (2), ALTALLAH S (2), BLACK GC (3), BONSHEK RE (4)

(1) Histopathology Department Mt, Manchester
(2) Ophthalmology Department Royal Eye Hospital, Manchester
(3) Medical Genetics Department St Marys Hospital, Manchester
(4) Histopathology Department MRI Royal Eye Hospital, Manchester

Purpose We present a case of a 29-year-old woman presenting with corneal rupture after minor trauma. This is the third person in the same family with corneal rupture and other features of brittle cornea syndrome (BCS). This is the largest known family with this syndrome and the age at corneal rupture is the oldest described.

Methods A 29-year-old woman presented with left corneal rupture evoked by minor trauma and received left evisceration and implant. Two of her younger sisters had also presented with corneal rupture when they were 3 and 4 years old. They had also blue sclera and several bone problems and they suffered ear problems. Another sister is known to have bilateral keratoconus, but no history of corneal rupture. All the injuries affecting the family members were provoked by minor trauma.

Results This is an interesting family with BCS. BCS presents with brittle cornea, blue sclera and red hair. Recurrent spontaneous corneal perforations, sometimes bilateral, are a feature. Other characteristics that have been described are hypermobility of the small joints, moderate skin hyperextensibility without excessive skin fragility, and hearing impairment.

Conclusion The major differential diagnosis of BCS is Ehlers Danlos syndrome type 6, since VI Ehlers Danlos is associated with poorer prognosis. It also needs differentiation from osteogenesis imperfecta. The exact genetic abnormality in BCS is yet to be discovered. A recent article has implicated chromosome 16 and offered some candidate genes, among them the PCOL1 which may have been implicated with collagen formation. BCS may be important in medical-legal cases as potential misdiagnosis of non-accidental injury may occur.

2126 / 244

Clinical management of congenital achromatopsia

PINELLO L (1, 2), MAZZAROLO M (1, 2), MAIMONE PE (1, 2), SLIPPEI A (3), CALDRONE P (4)

(1) Regional Pediatric Low Vision Center, Padua
(2) Pediatrics
(3) Pediatrics, Padua
(4) Robert Hollman Foundation, Padua

Purpose Describe the long term follow-up of children affected by congenital achromatopsia (CA). CA is a rare recessive inherited vision disorder (1:50000) and includes two clinical categories: complete CA and incomplete CA.

Methods Fourteen children (9M, 5F) were followed-up and a rehabilitation individual program was carried out. The assessment included: visual acuity (Teller, Lea Symbols, Snellen Charts), refraction, color vision (Farnsworth, Ishihara, Montessori), contrast sensitivity, visual field, slit lamp examination, fundus ophthalmoscopy, electrophysiologic tests (photopic, scotopic and flicker ERG and VEPs) and genetic examination.

Results Early onset of nystagmus, low vision and photophobia are first clinical manifestations of CA. The differential diagnosis includes Leber Amaurosis, and congenital nystagmus and albinism. Visual acuity in our patients is around 1/10 and it is worse for near. Refractive errors showed a distribution toward hyperopia (50%) and emmetropia (35%). Macular hypoplasia is present in all cases and electrophysiology showed a reduced or absent photopic ERG while scotopic system appears normal.

Conclusion Due to abnormal or totally absent cone function, which lead to macular hypoplasia, CA shows reduced visual acuity, absent/severely impaired color vision, nystagmus and photophobia. Low vision aids such as telescopic systems and magnification devices along with OCR/ICR software were prescribed after a carefully evaluation. Spectral filters were early prescribed to reduce photophobia and improve vision. The molecular analysis may confirm the diagnosis and give genetic counseling to the family. An early detection of CA is underlined by the importance to carry out a complete and precocious rehabilitative approach.
* 2131
The problem of non-adherence with medical therapy

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Purpose To discuss the topic of non-adherence with medical therapy.

Methods Adherence may be defined as the extent to which a patient's behavior coincides with medical advice. The success of long-term therapy in chronic diseases depends upon patient's adherence.

Results Unfortunately, published evidence suggests that poor adherence is the single most important problem in long-term medical therapy. The level of non-adherence is generally underestimated by clinicians. Improving adherence depends upon a successful doctor/patient communication but numerous other factors also influence adherence e.g. ease of medical therapy administration etc. Good adherence is critical for preventing disease progression.

Conclusion New approaches are needed to enhance adherence with chronic medical therapy in real life practice.

* 2132
Adherence with antiglaucoma therapy

CARASSA RG
Ophthalmology, University Hospital S. Raffaele, Milano

In recent years an increasing attention has been drawn towards the performance of drugs in the real world. Beyond the mere IOP lowering effect, the treatment success implies a number of issues regarding the acceptance and adherence to the therapy. Health outcome studies and studies on compliance showed poor persistency and adherence levels for glaucoma medical therapy with higher scores for prostaglandine analogues. Adherence is in fact linked to many variables: regimen factors (as complexity of treatment, tolerability, side effects), patient factors (as memory, motivation, knowledge), provider factors (as dissatisfaction or poor communication with the physician) and situational factors (as competing activities, change in routine). The choice of a therapeutic strategy capable of minimally influencing the patients' quality of life is crucial for optimal adherence and improving doctor/patient communication is essential in favouring patient's decision making about health-promoting behaviors.

* 2133
Patient reported problems and adherence to glaucoma medications in the United States and India

SLEATH B
University of North Carolina C. G. Shops for Health Services Research and School of Pharmacy, Chapel Hill (North Carolina)

Purpose The purpose of this research was to examine patient reported adherence and problems in using glaucoma medications.

Methods In the United States, 324 patients on adjunctive therapy who were seen at four geographically distinct ophthalmology practices participated. In India, 243 patients seen at a glaucoma clinic in an eye hospital in Southern India participated. All patients were asked to complete a survey before their visits with their ophthalmologists.

Results The U.S. sample ranged in age from 20 to 91 (mean age 68 years) and was 48% female. The India sample ranged in age from 19 to 90 (mean 63 years) and was 36% female. Sixty-one percent of the U.S. sample and 42% of the India sample reported one or more problems in using their glaucoma medications. Fourteen percent of the U.S. sample reported being less than 100% adherent in taking their glaucoma medications in the past week. Patients who had difficulty remembering to take their glaucoma medications and those who reported other problems or concerns were significantly more likely to report being less adherent. Six percent of the India sample reported being less than 100% adherent in the past week. Patients who reported difficulty squeezing the bottle and those who reported difficulty opening the bottle were significantly more likely to reported being less adherent.

Conclusion Patients in the U.S. and India reported many problems in using their glaucoma medications and in each country specific types of problems were related to patient non-adherence.

* 2134
Fixed combinations and adherence

HOLLO G
Semmelweis University, Budapest

Purpose To summarise information on adherence of glaucoma patients using fixed combinations of IOP lowering medications.

Methods Literary search.

Results Clinical experience as well as published studies clearly show that like compliance, adherence to prescribed medication is far not optimal in glaucoma patients. When using prostaglandin analogue eye drops QD adherence is better to the prescribed medication than in treatment periods on BID medication or in patients treated with BID topical medication. Combined prostaglandin/beta-receptor blocker medication is similar to prostaglandin analogue monotherapy in terms of number of application per day, and can substitute unfixed combinations consisting of a prostaglandin analogue plus a beta blocker. Thus, improved adherence to the combined medications given once daily is supposed to be similarly good as it is with prostaglandin analogues. However, little published data is available on this field.

Conclusion Combined prostaglandin analogue/beta-receptor blocker medication has a promise of good adherence because of the ease of administration and powerful IOP lowering, but for exact figures further adherence studies are necessary.
**2135**

Objective evaluation of adjunctive glaucoma therapy

ROBIN A

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**Purpose** To measure adherence with electronic monitoring in two populations of subjects: those using once-daily prostaglandin analogs (q.d.), and those on adjunctive medicine to the prostaglandin analog.

**Methods** Single-site, open-label, non-randomized, parallel, 60-day study. 62 open single glaucoma or ocular hypertension adult subjects currently using either a topical prostaglandin as sole ocular hypotensive medical therapy q.d. ("1-drug" subjects, n = 31) or a topical prostaglandin plus an adjunctive topical ocular hypotensive product either q.d., twice-daily (b.i.d.), or three-times-daily (t.i.d.) in the same eye(s) ("2-drug" subjects, n = 31). An electronic event medication monitoring device was used to record each opening of the bottle containing the ocular hypotensive medication. The main outcome measures were dosing errors (number of under or over-adherence events) and coverage (proportion of pharmacological duration covered by dosing) relative to ophthalmologist-prescribed regimen.

**Results** Adherence to the prostaglandins q.d. was good in both groups by all measures (<10% of subjects with >5 dosing errors and mean coverage of 97.2±0.1%). Adherence to the second medication in the 2-drug group was poorer (37% of subjects with >5 dosing errors and mean coverage of 85.6±12.6%). For the subjects on β-adrenoceptor antagonists, 24.8±18.4% of doses were taken at less than 10 hour intervals (over-adherence).

**Conclusion** The use of electronic monitoring provides more information than simple 'pill count,' bottle weighing methods, or prescription refill rate, as it incorporates a time component. We found that more complex dosing regimens result in poorer adherence, although q.d. drugs in a complex dosing regimen were found to have good adherence.

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**2136**

Update on a new randomised adherence study

TSIRONI S

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**Purpose** To present the design and aims of a new randomised adherence study, investigating the value of an intervention on the level of adherence with glaucoma therapy.

**Methods** Poor adherence with prescribed treatments significantly undermines treatment of glaucoma. In a new randomised study we will investigate the impact of intensive teaching and other measures on the level of adherence in consecutive newly diagnosed ocular hypertensive or glaucoma patients. A novel device, TDA, will be employed to facilitate adherence monitoring in the present study. This device may help physician in objectively assessing adherence with selective glaucoma therapy. This study will validate this monitoring device.

**Results** Documentation of the impact of intensive teaching on the level of glaucoma patients’ adherence.

**Conclusion** The results of this study may determine if adherence of glaucoma patients is modifiable by education and consequently, it will indicate if we should focus our efforts in this approach in the future management of glaucoma.
**2141**

The first consultation & photophobia in anterior segment disease  

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Purpose First consultation and anterior segment diseases associated with photophobia are described. Photophobia may be caused either by ocular or neurological disorders.  

Methods Principles of first consultation include differential diagnosis between ocular and neurological causes of photophobia. Detailed history for exposure to foreign bodies, allergens, systemic symptoms (e.g., arthritis) and physical exam should include a detailed ocular exam (including dilated fundoscopic and slit lamp exam if possible), eyelid eversion to rule out foreign bodies, fluorescein staining of the cornea to rule out abrasion, and intraocular pressure measurement is mandatory. In suspected neurological diseases, neurological workup is necessary.  

Results Almost any condition of the eye may cause photophobia, including dry eye, conjunctivitis, blepharitis, keratitis, corneal dystrophies, iritis, aniridia, albinism, glaucoma, optic nerve and retinal diseases. If anterior uveitis is suspected, targeted testing may include CBC, ESR, ANA, ACE level (sarcoidosis), RPR (syphilis), PPD, chest X-ray (TB/sarcoidosis), Lyme titers, Chlamydia cultures (Reiter’s syndrome), HLA-B27 assay, sarcoidal spine films (ankylosing spondylitis), and choroscopy (inflammatory bowel disease). If retinal dystrophy is suspected, electrophysiology is mandatory for the diagnosis. If optic neuritis is diagnosed, an MRI of the brain and orbits is indicated to evaluate for demyelination. Other neurological reasons include migraine, meningitis and SAH. A number of drugs can also cause photophobia.  

Conclusion Detailed history often gives good insight in differential diagnosis. However, differentiation between ocular and neurological disorders may be complex and should be based on detailed clinical examination and appropriate tests.

**2142**

Photophobia in ocular developmental disease  

MOORE T  
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ABSTRACT NOT PROVIDED

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**2143**

Photophobia in inherited retinal disease  

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Purpose To describe the phenotypes and genotypes of genetically determined photophobia.

Methods A case presentation format will be used to illustrate different genetically determined conditions leading to photophobia. Both clinical and electrophysiological phenotypes as well as genotypes will be discussed.

Results Phenotypes and genotypes of genetically determined diseases leading to photophobia are very different. An important distinction to be made is the one between stationary and progressive diseases. Indeed, such distinction is important as the visual outcome may differ considerably between different conditions.

Conclusion Genetically determined photophobia is diverse. Visual electrophysiology allows an important distinction between progressive and stationary conditions.

**2144**

The role of electrophysiology  

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(2) Institute of Ophthalmology, London  

Purpose To demonstrate the role of electrophysiological assessment in the investigation and management of the patient with photophobia, or whose vision is worse under bright lighting conditions.

Methods The ISCEV standard techniques for ERG, PERG, mfERG and EOG recording will be described.

Results Selected cases will be used to illustrate the value of the objective data provided by electrophysiology.

Conclusion The objective data provided by electrophysiological assessment is important to the assessment, diagnosis and management of this group of patients.
**2151**

Functional anatomy, innervation and immune regulation of the ocular surface

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(2) Research Lab, Dept. of Ophthalmology, Charité-Universitätsmedizin, Berlin

**Purpose** The functional anatomy of the ocular surface prominently contributes to the visual function of the eye. It is aimed to describe the complexity of this system.

**Methods** Own results on histological, immunohistological, ultrastructural and biochemical level in the human and the rabbit eye are discussed together with results from the literature.

**Results** The ocular surface in a functional view consists of the mucosal surfaces of the globe and lid (cornea & conjunctiva) and of its mucosal adnexa (lacrimal gland & lacrimal drainage system) and it also includes the lid apparatus required for eye closure and blinking in order to provide lubricants. Different functional systems interact to produce the intact functional anatomy. These are e.g. epithelial differentiation, secretion, lid motion and immune defence that together lead to the higher order function of wetting and comfort. Ultimately they allow the preservation of corneal clarity and visual function at the ocular surface level. These systems are governed by similar underlying regulatory mechanisms as e.g. growth factors, cytokines, hormones, neuromodulatory molecules and innervation which are all shown to be involved in ocular surface function and also interact through common signalling molecules. An important functional system is the immunological defence system of the ocular surface that is known as the Eye-Associated Lymphoid Tissue (EALT) and appears to have a central integrative role.

**Conclusion** Different functional systems interact at the ocular surface by interactions based on common signalling molecules, which may also allow psycho-neuro-immunological regulation. An integrative understanding of the physiological state may also give insight into potential interventions under pathologic conditions.

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**2152**

Principles of psycho-immunological relations – Do they apply to the eye?

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**Purpose** Symptoms and subjective feelings are often different, effectiveness of therapy is changing.In chronic diseases, like conjunctivitis we observed improvements by relaxing methods.In many diseases of the eye there are new aspects for the pathogenesis like immune-, hormonal forces or neurotransmitters. Studies points at psycho-neuro-immunological relations. Our question is, if this principles apply to the eye, too.

**Methods** In literature we found influences, both stress affecting the nerval and immune system, and disorders in the immune system affecting the nerval and psychic systems. In clinical observations we observed patients with dry eyes, their mental and autonomic nervous disorders.For dryness we observed the lipid layer, the tear film, the tear film break up time and tear meniscus, Schirmer’s Test, Fluorescein and Lissamin green staining. Severeness was determined by a score, built by the mean of measurements of dryness, all equated. For psychological problems we used records psychic diagnosis by ICD 10. Pearson correlation served for statistical analysis.

**Results** In 27/34 (79%) patients we could detect mental and behavioral disorders. Anxiety was found in 26/34 (76,5%); patients, depression in 18/34 (52,9%). For vegetative symptoms most patients suffered from stress or nervousness (24/34). The correlation for vegetative complaints to severeness of dryness was 0,57, significant. There was a trend in correlation of dryness to anxiety and depression. Relaxing methods improved the satisfaction to the treatments.

**Conclusion** Principles of psycho-immunological relations are applicable to dry eyes. Therefore relaxation methods should have good effects in dry eyes to support the local treatment.

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**2153**

Psychosomatics and implications on clinics of patients with blepharospasm

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**Purpose** To evaluate the possible causes of blepharospasm and the implications of clinical appearance.

**Methods** 31 patients of the Department of Ophthalmology, Medical University of Vienna, with Blepharospasm were included in the study. We evaluated form and intensity (Elston Score) of blepharospasm and influence of psychosomatics and physical disease.

**Results** 15 patients had unilateral and 16 bilateral blepharospasm. In 83.8% of cases symptoms were continuously tonic with tics and blinks. In 64.3% of patients the intensity of disease was slowly increasing, 38.7% of patients reported a sudden begin. Psychosomatic influence in aetiology was found in 45.1% of patients who reported about periods of massive psychological stress within the start of disease. In 25.8% of patients blepharospasm was related to other physical disease like facial nerve palsy, and in 29.1% of patients no reason for disease could be evaluated. Patients with psychosomatic aetiology of disease had a mean Elston Score of 4.15, the other patients felt significantly less limited in function with a mean Elston score of 4.8 (p<0.05). Patients with psychosomatic aetiology were statistically significant more under constant psychic stress, showed a greater tendency of obsessive behaviour and had less intact family structures (p<0.05).

**Conclusion** The present study clearly shows that blepharospasm often has a psychosomatic reason in its aetiology. This influence should not be neglected in the treatment of patients with blepharospasm.

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**2154**

Influence of bone marrow transplantation on the eye and psychopathology

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(2) Ophthalmology, Medical University, Vienna
(3) Psychiatry, Medical University, Vienna
(4) Bone Marrow Unit Medical University, Vienna

**Purpose** Introduction: Bone Marrow Transplantation because of Leukemia is followed by changes of the immunological system. Patients suffer from complaints of the disease and its consequences, like Graft versus host reactions. In ophthalmologic observations we often can assess chronic inflammations with dry eyes. These are induced by stress reaction, any kind of inflammation and any kind of irritations. Now we wanted to know, if there is a correlation of the ophthalmological changes, especially the dryness and the mental state, according to the bone marrow transplantation.

**Methods** 26 patients were observed, 11 of them in a follow up after 100 days. For ocular evaluations slit lamp observation were done. Severeness of Dryness was defined by a Sicca Score, a mean of results of Schirmer’s test, break up time, lipid layer thickness, and estimation of the tear meniscus. All results were equated. 0 for normal and 1 for worst possible result. Psychiatric symptoms were assessed by the symptom check list 90R (Derogatis) at baseline and follow up. We compared the results before and after the transplantation. For statistical analysis students T-test and the Pearson correlation by SPSS was used.

**Results** There was a significant aggravation of the dryness in one eye; and there was a correlation in parts of the records between the dryness and the mental state.

**Conclusion** Bone marrow transplantation is an event, which influences the immunological system, the tearing system and the mental state of health.
Our eyes are the sense organs which provide us with the greatest perspective of our world. Therefore they are deemed to be the most intellectual of our sense organs. But through our eyes we can understand only to a certain extent. We need the use of other senses which come together, stabilize and are rooted in our neurophysiological system. The eyes are an important door to the world which inspires and motivates to further discovery and invention. They create aspects of motivation dealing with perception, combinations of distant, nearby and internal senses which are the basis for increasing the depth of our knowledge and true understanding. We also want to emphasize the emotional side of those interna liked, neurophysiological "bio-psycho-representations" (Gerber/Reinelt 1989). Emotions are created within us - fear, joy, panic, visions, dreams and much more. This "Power of internal images" (Haether, 2006) is our partner on our way. Each psycho-therapy works at these basics to differentiate, to change these emotions with whatever motivation created them. In addition, we will show, based on 'Constructivism' (Foester, 1999), that in each of us there arise subjective realities, which are individual and not objective. But what happens when we are unable to see with our eyes? When one is blind or visually handicapped? As a result of these questions we want to describe our project 'Theatre for Everyone' in the Vienna Schauspielhaus with Arian Berg as director with Walther Schoen and Birgit Ecker in cooperation with the professors Gisela Gerber and Toni Reinelt as scientific co-ordinators from the University of Vienna. Conni Ederer und Clemens Desero are the student's co-ordinators. This project has been developed since 2005 for visually handicapped and blind people, enabling them to attend the theatre with the assistance of students.
2161  
Topical cyclosporine in the treatment of vernal keratoconjunctivitis in a Rwandan eye clinic: a prospective, randomized, double-masked, clinical trial  
DE SAEDTS  
UZ Gent, Gent

2162  
Structural and functional arterial properties in low-tension glaucoma  
HEIREMANS  
UZ Gent, Gent

2163  
Correlation between visual field defects and fitness to drive in glaucoma patients  
LELOUP E  
UZ Gent, Gent

2164  
Serum markers in screening for regional and distant metastases in conjunctival melanoma patients  
MISSOTTEN GS  
UZ Antwerpen, Antwerpen
2211
Microbiological culture and universal PCR yield after intravitreal injection of antibiotics in acute endophthalmitis following cataract surgery

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(2) Ophthalmology, University Hospital, Dijon
(3) Ophthalmology, University Hospital, Lyon
(4) Ophthalmology, University Hospital, Saint Etienne
(5) Microbiology, University Hospital, Lyon

Purpose To isolate bacteria after intravitreal antibiotic injection in acute endophthalmitis following cataract surgery; using cultures and bacterial PCR.

Methods We prospectively enrolled in a multicenter study 100 eyes of 100 patients (2004-2005) presenting an acute endophthalmitis following cataract surgery. Aqueous humor samples were collected before and after one intravitreal antibiotic injection (vancomycin and ceftazidim) for 53 patients while 19 patients underwent a vitreous tap before and after intravitreal antibiotic injections. Microbiological identification was performed using BHI (Brain Heart Infusion) medium and bacterial PCR.

Results In aqueous humor, microbiological cultures associated with bacterial PCR identified a bacterium in 26.4% of cases after intravitreal antibiotic therapy. Positive cultures before treatment became negative after antibiotic treatment in 33% whereas initial positive PCR before became negative after antibiotic treatment in 35%. However PCR allowed the recognition of previously unidentified bacteria after antibiotic injections in 8% of patients. In vitreous samples, a bacterium was identified in 70% of cases before and in 81.2% after intravitreal injection. After antibiotic injection, initially positive cultures and PCR became negative in 90% and 7% of cases, respectively.

Conclusion A single intravitreal antibiotic injection is not sufficient to eradicate the pathogens responsible for acute post-operative endophthalmitis. Bacterial PCR is useful for the microbiological identification in vitreous samples after intravitreal injections of antibiotics.

2212
Impaired cytokine production of tumor infiltrating lymphocytes in a model of primary intraocular B-cell lymphoma

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(2) Cordeliers Research Center, Paris

Purpose The immune microenvironment is thought to play a major role in promoting tumor growth. To evaluate the role of this microenvironment in Primary intraocular lymphoma (POL), condition and characterize the cytokine polarization of infiltrating T-lymphocytes (TIL), a murine model of intraocular B-cell lymphoma was developed in immunocompetent hosts.

Methods Intravitreal injection of a syngeneic lymphomatous B-cell line in immunocompetent mice was performed. Clinical, histological and flow cytometric analysis were performed in order to characterize the tumoral invasion and the immune infiltration. Cytokine production of ocular cells was investigated by RT-PCR and fluorescent immunoassay with or without stimulation by anti-CD3 plus anti-CD28 antibodies.

Results Intraocular lymphoma developed in all eyes injected by lymphomatous B-cells. Up to 75% of living cells were T-lymphocytes. Cytokine profile analysis of the supernatant of ocular cells cultured ex vivo demonstrated the presence of IL10, IL6, IFN, and TNF. Stimulation with anti-CD3/anti-CD28 antibodies increased the IFN level, and led to the induction of IL2, production, completing the type 1 (Th1/Tc1-like) pattern of cytokine expression observed. IL6 and IL10 levels were not modified. IL12p70 and IL-2, potent Th1 or Th2 differentiating factors, were undetectable even after stimulation.

Conclusion Our results suggest that TIL from intraocular B-lymphomas are characterized by a Th1/Tc1 like profile that could be partially inhibited in vivo. These data raise the possibility of an in situ T-cell immunostimulation in order to reactivate the Th1/Tc1 lymphocytes and improve the intraocular anti-tumoral immunity.

2213
The antilymphangiogenic effect of aqueous humour

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Purpose The cornea, which is free of blood and lymphatic vessels bathes on the endothermal site in the Aqueous humor (AqH). Because of the direct contact of AqH to the cornea, we wanted to know whether it could prevent ingrowths of blood and, especially, lymphatic vessels into the cornea.

Methods In vitro: quantifying lymphatic endothelial cell (LEC) proliferation with a colorimetric (Brdu) proliferation ELISA with 5 or 20 µl of human AH and as control EGM-2MV medium. In vivo: murine model of inflammatory corneal neovascularization; treatment group (n=5) received 3µl of murine AH once a day at surgery and for the following three days (control saline solution). After five days, mice were sacrificed. Corneal flat mounts were stained with LYVE-1 as a specific lymphatic vascular endothermal marker and CD31 as panendothelial marker. Morphometry of blood and lymphatic vessels was performed with the image analysis software analySIS/B.

Results Human AH inhibited the proliferation of the lymphatic endothelial cells in vitro in a dose dependent manner (p=0.01847; x; 20µl: p = 0.0001). The topical application of aqueous humour inhibited the outgrowth of blood vessels significant (P = 0.0186) in comparison to the control. The outgrowth of lymphatic vessels was also inhibited significant (P = 0.0002).

Conclusion AH inhibits the proliferation of lymphatic endothelial cells in vitro and the outgrowth of blood and lymphatic vessels in the cornea in vivo. Tissues like the cornea, the iris and the ciliary body are naturally free of blood and, moreover lymphatic vessels. Therefore, the aqueous humour seems to play an important role to avoid the ingrowths of blood and lymphatic vessels into the anterior chamber and its adjacent tissues.
**2215**
The Uveitis subtype associated with Spondylarthropathies varies regarding to gender and the type of Spondylarthropathy involved

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2. Interdisciplinary Uveitis Center, Internal Medicine, Heidelberg

**Purpose**
Inflammatory bowel disease (IBD) and Spondylarthropathies (SpA) are said to be associated with distinct types of Uveitis. We compared Uveitis subtypes in patients with IBD and SpA, as well as in different SpA subtypes.

**Methods**
We identified 162 patients with SpA (AMOR classification): 93 Ankylosing Spondylitis (AS), 43 with undifferentiated (u)SpA, 10 reactive Arthritis, 12 Psoriatic Arthritis (PsA), and 5 enteropathy associated (eSpA) and their Uveitis (SUN classification).

**Results**
Patients with AS were male (67%) and HLA-B27+ (84%) showing typical unilateral or alternating anterior Uveitis with sudden onset in 77%. Still, 23% (21) showed bilateral anterior Uveitis. In this subgroup females predominated with 55%. They were HLA-B27+ in 80%. Patients with uSpA were male (60%) and HLA-B27+ (82%), but only showed typical Uveitis in 58%. A higher number of other Uveitis subtypes was found: 27% showed bilateral anterior and 15% other Uveitis subtypes. Again there was a female predominance, without change in HLA-B27 positivity (79%). A different pattern was found in the PsA cohort, which was female (58%) and HLA-B27 was infrequent. Here 25% of the patients had typical Uveitis, whereas 17% showed bilateral anterior and 58% other subtypes of Uveitis. Patients with eSpA were female in 80%, and HLA-B27 was less frequent (40%). None of the eSpA group had typical Uveitis, 80% a bilateral anterior and 20% other Uveitis subtypes.

**Conclusion**
The frequency of a ‘typical’ Uveitis associated with SpA in our cohort varied from 77% to 0%, decreasing from AS to eSpA. We see a high number of other Uveitis subtypes, with a female predominance. Maybe women with SpA have a higher risk for atypical Uveitis.

**2216 / 233**
Epidemiology of severe uveitis in France: a prospective multicentre study

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**Purpose**
To determine the characteristics and prevalence of severe uveitis, referred to university referral centres (URC) in France during a 3-month period.

**Methods**
All new cases of uveitis referred between January and April 2006, to 31 specialized URC, for diagnostic or therapeutic management were prospectively reviewed. Characteristics of ocular inflammation were reported at the end of the first examination on a questionnaire.

**Results**
527 patients were finally included. Median age was 44.1 (range 1.95) and the sex ratio was 1. The majority of patients (60.92%) were referred by their ophthalmologist and no previous episode of uveitis was noted in 62.75% of cases. Acute-onset uveitis was present in 68.22% of cases. Anterior uveitis was the most common type of presentation (54.7%) followed by panuveitis (21.3%). Complications at referral included a visual loss < 20/100 (20%), posterior synechiae (21.6%), secondary glaucoma (15.8%), cataract (7%), macular edema (7.8%) and retinal necrosis (1.7%). An infectious entity was suspected in 27.8% of cases. Among these patients, a viral infection was suspected in 11.8% of cases followed by toxoplasmic retinochoroiditis in 10.2% of cases. A noninfectious etiology was proposed in 48.6% of cases, including B27-like uveitis in 15.5% of cases.

**Conclusion**
Even though classical forms of acute anterior uveitis are still referred to tertiary eye care centres, severe presentations of posterior or panuveitis should be managed properly. Initial ophtalmological examination is a key step for the diagnostic and therapeutic management of these patients. An infectious etiology may be easily suspected and should be excluded before the further use of corticosteroids or immunosuppressive drugs.

**2217 / 234**
Intermedial uveitis: clinical course in ten years follow up

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**Purpose**
Intermedial uveitis (IU) is a chronic ocular disorder of unknown origin with frequent development of macular edema. The long term course of IU is not yet known and is a subject of this study.

**Methods**
Retrospective study of 28 patients with IU (12 male, 16 female, 49 affected eyes) in whom a follow up of at least 10 years starting with the onset of IU was available.

**Results**
The average age at the onset of IU was 37. Five patients had an associated systemic disease (2 sarcoidosis and 3 multiple sclerosis). At onset bilateral involvement was observed in 20/28 (71%) patients. Visual acuity (VA) of more than 0.8 was noted in 19/48 (39%) affected eyes, and VA of less than 0.1 in 2/48 (4%) affected eyes. Systemic treatment with immunosuppressant drugs was required in 7/28 cases (25%) and 13 eyes underwent one or more surgical procedures (cataract surgery in 10 eyes, pars plana vitrectomy in 2 eyes, scleral buckling in 1 eye). At ten-year follow-up VA of more than 0.8 was noted in 22/49 (45%) affected eyes, and VA of less than 0.1 in 10/49 (21%) affected eyes. The causes of visual loss included predominantly cataract, macular edema and vitreous opacities. Moreover, during the ten-year follow up intraocular inflammation diminished in 10/28 (36%) patients. The mean time to remission was 5.4 years (range 2.12 years). During the follow up, systemic disease sarcoidosis and multiple sclerosis manifested in two additional patients.

**Conclusion**
The main complication of IU consisted of cataract, followed by macular edema and vitreous opacities. Within ten-year follow-up one third of patient with IU achieved the long term remission of the intraocular inflammation.
**ABSTRACT NOT PROVIDED**
Myopia genetics: lessons from other complex diseases

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Purpose To discuss the lessons for myopia genetics that can be learnt from studies of other complex diseases.

Methods Literature review.

Results The sibling recurrence risk for high myopia (Ks > 10) suggests that the condition is a complex disease. Other complex diseases thus provide insight into the likely role of genetics in the aetiology of high myopia and the research strategies likely to be successful in identifying the genetic variants that confer susceptibility to it. In Mendelian disorders, there is a one-to-one relationship between a particular (“mutant”) allele at a particular locus and the development of the disease phenotype. In complex diseases, such “high penetrance” alleles are considered to be at one end of a spectrum of genetic effect size (alleles of large effect). Where they occur, alleles of large effect will tend to swamp other genetic and environmental contributions to the phenotype, and thus they typically produce classical dominant or recessive patterns of disease transmission in pedigrees. However, the large effect size of these alleles puts them under intense selection pressure, and thus they tend to be rare in the population (an exception being in late-onset diseases, such as age-related macular degeneration, where alleles of large effect have been found to contribute most of the population attributable genetic risk). Alleles of moderate effect do not show the one-to-one relationship between genotype and phenotype seen for alleles of large effect. Instead, they increase the likelihood of disease affection in a probabilistic manner (although the nature of this increase can be complex, e.g. dependent on the genotype at other loci, or to exposure to environmental stimuli). Alleles of moderate effect can be important causes of disease at the population level, despite their modest individual effect, since they can rise to high levels (by escaping selection pressure). In this presentation, I will review recent progress in mapping alleles of moderate effect in common complex diseases, in humans and animal models.

Conclusion The identification of risk alleles for common complex diseases can disclose the involvement of previously unanticipated genes or pathways (e.g. the association between autophagy and Crohn's disease).
**2231**

Retinal photocoagulation: clinical aspects

BRETT

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ABSTRACT NOT PROVIDED

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**2232**

The effect of laser on retinal oxygenation (animal studies)

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**Purpose** To discuss the effect of laser on the retinal oxygen distribution, in experimental animal models.

**Methods** Transectal and periretinal O2 measurements were obtained by O2 sensitive micro-electrodes in anesthetized animal models. The effect of laser photocoagulation on the oxygen periretinal and transectal distribution and retinal oxygen consumption, within normal and post experimental ischemic micro-angioptathies retinas, were calculated.

**Results** Laser photocoagulation, resulting to pigment epithelium and photoreceptors layers disruption, reduces the outer retinas demand for O2 and nutrients. Transectal PO2 measurements confirm changes of the transectal PO2 gradients distribution, O2 diffusion from the choord, non consumed by the outer retinal layers reaches the inner retina, resulting to PO2 raise in periretinal areas and in the inner healthy retinal layers of experimental animal and relieve retinal hypoxia in experimental BRVO in mini pigs and cats. Improvement of inner retinal oxygenation affect the retinal circulation resulting to retinal vessels construction and restoration of retinal blood flow regulation.

**Conclusion** Laser photocoagulation improves the inner retinal layers oxygenation, thus reversing tissue hypoxia and improving the retinal blood flow regulation in retinas with ischemic micro-angioptathies. Reversal of retinal tissue hypoxia and restoration of retinal blood flow regulation potentially affect the evolution of the macular edema related to ischemic microangiopathies.

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**2233**

Retinal oximetry in humans: effect of laser

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**Purpose** According to theory, laser treatment of the retina decreases the demand for oxygen and increases available oxygen for the remaining retinal tissue: Animal studies have shown that preretinal or intraretinal partial pressure of oxygen (PO2) decreases following experimental branch retinal vein occlusion (BRVO) and the PO2 rises again following laser treatment. Confirming the beneficial effect of laser on retinal oxygenation in humans has been difficult because practical non-invasive technology for oxygen measurements has been lacking. Our research group has studied retinal oxygenation in humans with retinal vein occlusion.

**Methods** The retinal oximeter is composed of a fundus camera, beam splitter and a digital camera. It yields fundus images with isochromatic and iso-isoluminant wavelengths of light simultaneously. Custom-designed software analyses the images and estimates haemoglobin oxygen saturation in retinal vessels. Measurements have been made on 25 patients with retinal vein occlusion, in some cases before and after laser treatment.

**Results** In the 5 patients with BRVO, who were measured both before and after laser, the oxygen saturation in affected vessels rose from 45±10% (mean±SD) before laser treatment to 53±6% after treatment (p<0.0041). Cases of hereditary central vein occlusion have shown low oxygen saturation before laser and increased oxygen saturation after laser.

**Conclusion** The preliminary data indicates that, in some cases, low oxygen saturation is measurable in vessels affected by retinal vein occlusion and that the saturation increases after laser. In some cases, notably where clinical signs are mild, the saturation appears to be normal even if no treatment has been applied but further studies are needed to establish the variability.

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**2234**

Physiological effects of vitrectomy and retinal laser treatment explain their clinical effects: Vitrectomy and laser treatment both affect retinal oxygenation and benefit ischemic retinopathies

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Clinical studies have established that retinal laser photocoagulation on one hand and vitrectomy on the other have a positive clinical effect on the development of diabetic retinopathy and other ischemic retinopathies. For both reducing new neovascularization and macular edema. The clinical effect of laser and vitrectomy is related to the physiological effect of these treatment modalities. Both improve retina oxygenation, but through different mechanisms. Laser treatment destroys some of the photoreceptors, reduces the oxygen consumption of the outer retina and allows oxygen to diffuse from the choord to the inner retina. In vitreous surgery the viscous vitreous gel is replaced by low viscosity water. According to the Stokes Einstein equation viscosity is inversely related to the diffusion coefficient. Diffusion (and convection) of any substance is many times higher in water than vitreous gel, and this includes oxygen, growth factors and drugs. In the vitrectomized eye, oxygen diffuses more easily from well perfused to hypoxic areas of the retina. At the same time growth factors are cleared from the retina more rapidly than before and may indeed be transported to the anterior segment and produce neovascularization. Laser and vitrectomy reduce retinal hypoxia and thereby reduce the production of hypoxia induced growth factors such as VEGF. Vitrectomy also clears VEGF away from the retina at a more rapid rate than when the vitreous gel is in place. VEGF and other growth factors influence both neovascularization and capillary permeability, and the latter influences the osmotic balance between blood and tissue and the osmotic arm of Starling’s law. In addition, improved oxygenation reduces the hypoxia-induced vasodilatation and this decreases capillary blood pressure and reduces edema formation according to the hydrostatic arm of Starling’s law. By understanding the physiological effects of laser and vitrectomy, we can understand the mechanisms of the clinical effect. Classical laws of physiology and physics help complete the picture and give us an understanding of the nature of these treatment modalities and how they may be combined with other agents. For details and references please see Steffansson E. Survey of Ophthalmology 2006.
2241
Optimal stimulus size maps in the primary visual cortex revealed by optical imaging in cats

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Purpose It is well known that the responses of cells in the primary visual cortex depend on stimulus size. While the stimulus-size dependency has been well documented at the cellular level, nothing is known about its consequences on global functional maps.

Methods Optical imaging of intrinsic signals in the primary visual cortex was carried out in anesthetized cats. Stimulation consisted of 0.75 to 0.1 cycles per degree square-waves drifting in 8 directions at 2 to 4 Hz and were presented monocularly. Responses were obtained for different stimulus diameters (1 to 50 deg. and a full screen condition).

Results The minimal visual stimulation necessary to activate areas 17 and 18 was around 3 and 6 deg. in diameter respectively. The activation area of cortex (10-30 mm2) was dependent of the eccentricity (0 to 30 deg). The psidewme measure of the signal magnitude in this area showed a modular organisation uncorrelated with the orientation map and stable in time. Half of the pixels had a maximum activation for the full screen stimulation (full field facilitation) and the other half attained their maximum for diameters about 15 and 30 deg of diameter in area 17 and 18 respectively (full field suppression). The suppression by the full screen stimulation was around 30% in both areas.

Conclusion Thus, the maximum activation revealed by optical imaging necessitates the stimulation of a much larger spatial area than that observed with single cells. This difference is likely due to the fact that this method reflects in- and out going signals and reveals activity of adjacent neurons being part of intra-cortical and thalamo-cortical circuits. Sapp NSERC and CIHR.

2242
Mathematical analysis of the cone ERG photopic hill: Clinical applications

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Purpose With brighter stimuli, the photopic ERG B wave increases to a maximal value and then decreases to a plateau, a feature known as the Photopic Hill (PH). Recently, a mathematical model combining a Gaussian (GF) and a Logistic Growth (LGF) functions was developed to fit the PH (Hamilton et al., Vision Research, in press). We examined if this equation could help us sort out selected retinopathies.

Methods We compared PHs (baseline: 30 cm-r; intensities: -0.8 to 2.84 log cd/deg-s) obtained from normals (N=10) and patients (N=20) affected with Congenital Stationary Night Blindness (CSNB), Congenital Postreceptorial Cone Pathway Anomaly (CPCPA), and Retinitis Pigmentosa (RP) with the GL ratio (GL = GB/Gv(B+Vmax)) were GL and Vmax represent the amplitude of the Gaussian and logistic (Vbmax) functions respectively.

Results The normal GL ratio is 0.60 ± 0.08 (mean ± 1SD) compared to -1.0 in CSNB (almost pure GF) and 0.32 ± 0.08 in CPCPA, reduced GF (p<0.05) and normal GF (p<0.05) patients. Six of the 8 RP patients had a GL ratio above 0.5 (mean GL = 0.70 ± 0.19) and 2 below (0.28 and 0.41). Of interest, while in some retinopathies, a decline in GB and Vmax occurred with disease progression (longitudinal and transversal comparisons), it did not always modify the GL ratio.

Conclusion Human PH can be dissected into two distinct and concomitant phenomena each represented by its own equation. Although the retinal origin of the GF and LGF awaits to be confirmed, use of this mathematical approach appears to add valuable information that will further refine the diagnosis of retinal disorders affecting the photopic (cone) pathway. Supported by CIHR and Resea Vision.

2244
Optical inhibition of axial myopia progression

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Purpose The Dual-focus Inhibition of Myopia Evaluation in New Zealand (DIMENZ) clinical trial tests the efficacy of myopic retinal defocus in slowing myopia progression.

Methods Dual focus (DF) soft contact lenses have a central correction zone and peripheral zones that cause 2.00D of simultaneous myopic retinal defocus during distance and near viewing. Children (12-14 yrs, n=40, mean refractive error: -2.70D) were fitted with a DF lens in one eye and a single vision distance (SVD) lens in the fellow eye. After 10 mos. wear, lens assignment was swapped between eyes and the lenses worn for a further 10 mos. Accommodation was measured using an open field autorefractor. Myopia progression was monitored using cycloplegic autorefraction and partial coherence interferometry every 5 mos.

Results Visual acuity and contrast sensitivity of eyes wearing DF & SVD lenses were not different (p=0.209 & 0.157). At the 12 mos. mark, a DF lens was worn with either a single vision near (effective add = -2.50D) or a SVD lens in the fellow eye, children accommodated normally to a target at 40 cm. Preliminary results for 10 children after 10 mos. showed that the mean (+ 1 SE) increase in myopia and axial length in eyes wearing the DF lens (-0.45 ± 0.40D & 0.08 ± 0.07mm) were significantly less (p<0.0001 & 0.0002) than in eyes wearing the SVD lens (-0.79 ± 0.41D & 0.21 ± 0.11 mm). Thus, DF lenses had a 12 mos. adjusted treatment effect of 0.40D & 0.15 mm.

Conclusion DF lenses provide normal acuity and contrast sensitivity. They do not alter accommodative response, so provide myopic retinal defocus at distance and near Myopia progression is slowed significantly in eyes wearing DF lenses, suggesting that myopic defocus acts as an anti myopiogenic stimulus.

Commercial interest
Macular abnormalities and the Rarebit Fovea Test

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Purpose To describe Rarebit Fovea Test (RFT) findings in healthy subjects with normal best corrected visual acuity (BCVA) and macular abnormalities, observed in fundus photographs.

Methods Forty-two healthy subjects, mean age 60 years (SD 10), were recruited as controls in a previous study. Selection criteria were BCVA ≥1.0, refractive error within ±0.6 D, no ophthalmic or systemic disease. Reported here are RFT and fundus photography findings, and retinal thickness measurement values using optical coherence tomography (Stratus OCT, Carl Zeiss Meditec Inc.).

Results Fifteen of the 42 subjects had visible macular changes in the fundus photographs; 10 had drusen and 5 pigment epithelium defects. Mean RFT mean hit rate (MHR) was 99 ± 2%. Two subjects had MHR 91% and 96%, respectively, i.e. below the pre-defined limit for normality (97%). The subject with a MHR of 91% had drusen, the one with 96% had normal fundus. OCT values were within normal limits in all subjects (center zone mean 212.41 µm, SD 19.35, range 166-257, inner perifoveal zone mean 238.19 SD 13.34, range 258.3-308.3 and outer perifoveal zone 241.53 SD 11.54, range 218-267.8). In the subject with RFT MHR 91% corresponding values were 228 µm, 288.5µm and 249.3 µm and in the subject with RFT MHR 96% 211 µm, 279.8 µm and 231.8 µm, respectively, well inside the normal range.

Conclusion The Rarebit Fovea Test may be useful for discriminating between age-related macular changes with and without detrimental effects on visual function.

Fixation stability during the Rarebit Fovea Test

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Purpose To evaluate the fixation stability in normal subjects during the Rarebit Fovea Test (RFT). The RFT is a computerized test designed to evaluate the integrity of the central retinal detector matrix.

Methods 11 healthy subjects with visual acuity ≥1.0, were examined, using the standard RFT setup, i.e. stimulus presentation on a 17 inch computer screen at a viewing distance of 2 m. The test principle is to briefly present one or two very small (≤0.5") and bright dots in the central 4 x 3" visual field. The subject responds by clicking the computer mouse once or twice. The test time is approximately 90 sec and audio-stimulation is given each time the fixation target is rotated in order to enhance central fixation. Fixation stability was measured during the RFT test using the PowerRefractor® (Purkinje image eye-tracking). One eye in each subject was tested. All measurement data were analyzed using SPSS and Matlab.

Results All subjects had normal RFT test results, i.e. close to 100% of the presented dots were perceived. 97% of the testing time fixation was maintained within 1.38° and 100% of the time within 2.76°.

Conclusion Normal subjects appear to maintain stable fixation during the RFT. Further studies of patients with minor macular changes are underway.
The dynamic cell biology of extraocular muscles

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Extraocular muscles (EOMs) have unusual cell biological properties that should be considered when trying to understand control of eye movements. The myosin heavy chain (MyHC) isoform composition of these muscles is extremely complex compared to limb skeletal muscles with co-expression of up to 8 isoforms in individual myotendons. In addition, contrary to most limb skeletal muscles, individual muscle fibers do not run from tendon to tendon. As a result, neuromuscular junctions are dispersed along the muscle length. Individual myotendons in the EOM continuously remodel, adding and subtracting myonuclei in normal, uninjured adult muscle fibers. The extraocular muscles thus represent extremely dynamic structures, from the cell biological point of view, and these characteristics rapidly and continuously respond to different stresses placed on the muscles. It is predicted that changes in patterns of eye movements would in turn result in changes to the structure of individual myotendons within those extraocular muscles. The continuous remodeling that occurs in normal adult extraocular muscles has important implications for understanding the control of eye movements.

The potential role of sensory receptors in ocular movements

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Purpose To study the comparative picture of proprioceptors in extraocular muscles (EOMs).

Methods EOMs of monkeys, sheep, cats, guinea pigs, rabbits, rats and humans were studied histologically, with emphasis on the neuromuscular arrangements. The various species were chosen for their diversities with respect to eye positions within the skull and visual requirements, in order to achieve a broad basis for comparison. All samples were prepared following standard histotechnical techniques.

Results Muscle spindles (MS) are distributed in EOMs of sheep and man, receptors of the latter species being of a more unconventional morphology. Nerve endings with sensory features and myotendinous cylinders (MTCs) are present in the myotendons of all studied species, including guinea pig and rat. MTCs are also exposed in human EOMs of all ages, including infants. Golgi tendon organs (GTOs) are present in the myotendons of sheep and monkey. The density of proprioceptors varies between individual animals.

Conclusion The receptor population is found to be species dependent, suggesting a variation in the nature of proprioception between animal groups. The inconsistency in distribution between individual animals implies the sensory contribution also to be highly variable. The peculiar morphological features of MSs in human EOM, as well as the lack of GTOs and other potential sensory structures, suggest MTCs to possibly be the most significant proprioceptive contributors in humans. Other animal ocular systems, some of which encompass the full range of ocular proprioceptors, must therefore arguably be of questionable value as models for understanding human ocular motility.

Morphological variations found in human extraocular muscles and their functional implications

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Purpose The variation in ocular motility observed in healthy individuals may be a reflection of the morphological differences found in the neuromuscular arrangement of human EOMs. Studies of these variations may enhance our understanding of the etiology of some ocularmotor anomalies.

Methods Muscle samples were collected from EOMs of 10 healthy individuals aged 1 day to 83 years. Four samples were also obtained following strabismus surgery. All specimens were prepared for light and electron microscopy by standard histochemical techniques.

Results The complement of multiply innervated muscle fibers constituted between 10-30% of the fiber population and varied between individuals of all age groups. The lowest numbers were observed in the muscles samples obtained from strabismus surgery. The number of tendon receptors, previously found to be associated with the multiply innervated muscle fiber, was scarce in the infant material. Age-related changes such as lipohasexin, fragmentation of myofilaments, and anomalous endplates where found to increase with the chronological age of the patient.

Conclusion Individual differences in the morphological arrangement of human EOMs do occur in muscle samples from all age groups. Although the material is limited it is tempting to conclude that factors such as variations in the composition of muscle fibers and complement of proprioceptors may contribute to the development of certain ocularmotor anomalies. The diversity in the neuromuscular architecture increases with age as more and more muscle fibers are subjected to changes. These age-related changes arguably account for some of the changes in motility observed in the elderly.

Orbicularis oculi muscle and eyelid movements in health and disease

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Purpose To study facial motor control and motor learning in health and disease using eyelid movements as paradigm.

Methods Different types of recording techniques (electromyography, electromagnetic field, MDMT, IMRE) were used to monitor changes in the kinematics of eyelid movements and to determine guided central and peripheral neuronal changes in time.

Results Timing and control of eyelid movements, start time of eyelid movement, during blinking are regulated at three levels, the brainstem, the motor cortex and the cerebellum. In Bell’s palsy patients start times of eyelid movement are disturbed at onset. A 1.5 year longitudinal study in Bell’s palsy patients indicated that recovery of orbicularis muscle activity and of eyelid movements at the affected side starts 3 months after onset and start times at both sides were reset. Centrally, an ongoing increased activation is observed in the representation field of the motor cortex till the end of the study. In cerebellar ataxia patients start times of eyelid movement are disturbed and the ability for motor learning ability decreases in time.

Conclusion Eyelid movements are excellent tool to study neuronal plasticity during impairments and improvements of eyelid function.
Neuromuscular junctions of human extraocular muscles: looking for new tools for medical treatment of strabismus

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Purpose To investigate the composition of the neuromuscular junctions (NMJ) of human extraocular (EOM), in particular with respect to gangliosides GQ1b, GT1a, GD3, GD1a and GD1b.

Methods Samples from eleven EOMs and seventeen skeletal muscles were processed for immunocytochemistry with highly specific monoclonal antibodies (Abs) against gangliosides GQ1b-GT1a-GD3, GD1a and GD1b and other NMJ markers (extracellular matrix, cytoskeleton) as well as acetylcholinesterase, in serial sections. The neuromuscular junctions of the muscle spindle fibers found in skeletal muscles were also studied.

Results Abs against gangliosides GQ1b-GT1a-GQ1b strongly labeled the neuromuscular junctions (NMJs) in the human EOMs and muscle spindles, but not those of skeletal muscle fibers. Staining was observed in both end plate and in en gruppe motor endplates in the EOMs, but there was a particular preference for the NMJs of fibers in the orbital layer and of multiply innervated fibers.

Conclusion These gangliosides have been identified in the sera of patients with Miller Fisher syndrome, a condition characterized by ophthalmoplegia, ataxia and areflexia. Here we show a differential distribution of these gangliosides in human NMJs, and among the different fibers in the EOMs, providing both the molecular basis for the selective involvement of the EOMs in Miller Fisher syndrome and a potential new tool for the development of medical agents for the treatment of strabismus. Further studies are underway to correlate fiber types in the EOMs with the presence of additional gangliosides and other NMJ markers.
2261 Overcoming the barrier of the neural retina for the delivery of non-viral gene complexes to the retinal pigment epithelium

PEETERS L
UZ Gent, Gent

2262 Role of VEGF-isofoms in pathological angiogenesis

VAN BERGENT
UZ Leuven, Leuven

2263 The role of the VEGF family in anti-angiogenesis and neurodegeneration in the retina

VAN DE VEIRE S
UZ Leuven, Leuven

2264 Ex vivo expansion of human limbal epithelial stem cells for the treatment of corneal diseases with limbal stem cell deficiency

ZAKARIA N
UZ Antwerpen, Antwerpen
SIS: A Report from the International Dry Eye Workshop (DEWS)

2311
Definition & classification of dry eye

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Purpose To provide a contemporary definition and comprehensive classification of dry eye disease.

Methods A literature search and a consensus process involving members of the Definition and Classification subcommittee and the DEWS membership.

Results A new definition of dry eye was developed, reflecting the current understanding of dry eye disease. The committee recommended a three-part classification comprising: i. Aetiology - accommodating the multiple causes of dry eye, ii. Mechanism, indicating how the several causes of dry eye may act through a common pathway; and, iii. Severity, offering a rational basis for therapy in an individual case. In the course of discussions certain principles were established, such that ocular surface health is maintained by a functional unit whose integrity is essential for surface homeostasis. Interruption of the regulatory feedback loops, for instance by reducing the sensory drive to lacrimal secretion, is a potential risk factor for dry eye. Further, it was stressed, that any form of dry eye may interact with and exacerbate any other forms of dry eye, as part of a vicious circle, and that, in time, a change in tissue reactivity may be established leading to a disease which is self-perpetuating in the absence of its original trigger.

Conclusion The guidelines presented are not intended to override the clinical assessment and judgment of an expert clinician but should prove helpful in the conduct of its clinical practice and research.

Commercial interest

2312
The epidemiology of dry eye disease

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Purpose The goals of the Epidemiology Subcommittee of the 2007 Dry Eye Workshop were to evaluate and present knowledge on the epidemiology of dry eye disease, to summarize dry eye risk factors and and to review and assess dry eye questionnaires.

Methods Review of the published English language literature of dry eye epidemiologic and clinical studies which included dry eye questionnaires and which used rigorous methods was performed. Dry eye questionnaires were required to have been used in clinical trials or epidemiologic studies, undergone psychometric evaluation and be publicly available.

Results Based on large epidemiologic studies, the age-specific prevalence of dry eye ranges from 5 to 35%. Different operational definitions of dry eye are likely to be responsible for some of the disparity between studies. There are very little data on the natural history and incidence of dry eye. The public health importance of dry eye is heightened by the high proportion of older people affected and the aging of the world population. Dry eye can have a deleterious impact on ocular and general health and quality of life and visual function. Both direct and indirect costs exist for dry eye. Major risk factors for dry eye include age, female sex, certain medications and diseases, incisional refractive surgery, nutritional intake of essential fatty acids and disturbance in sex hormones due to a variety of causes.

Conclusion There is a need to expand epidemiological studies to more geographic regions, to include multiple races / ethnicities and to establish a consensus on diagnostic criteria for epidemiological studies. Dry eye questionnaires should be responsive, reproducible, with a specified recall period, validated and appropriate for the use. Vision targeted quality of life and prevention strategies warrant further study.

2313
Diagnostic methodology

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Purpose The Diagnostic Methodology Subcommittee of the International Dry Eye Workshop (DEWS) identified tests used to screen, diagnose and monitor dry eye disease, and established criteria for test performance and considered the utility of tests in clinical settings.

Methods The Subcommittee created a database of tests for diagnosis and monitoring dry eye, each test was compiled by an expert in the field (supporter) and presented within a standard template. The completed database is searchable by an alphabetical list of test names, as well as by functional groupings, for instance, tests of aqueous dynamics, lipid functions, etc. The templates can be accessed on the website of the Tear Film and Ocular Surface Society (www.tearfilm.org).

Results The limitations of the effectiveness of tests in the diagnosis of dry eye and the factors which affect the sensitivity, specificity and predictive value of tests are discussed. Ways in which sensitivity and specificity can be optimised are illustrated in relation to diagnosis by tear hyperosmolarity.

Conclusion A number of diagnostic tests, used singly and in combination, are available for the screening and diagnosis of dry eye disease; these are presented within a standard template prepared by DEWS.

2314
Clinical trial methodology in dry eye disease

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(3) Harvard Medical School, Boston

Purpose Clinical trials in dry eye disease have often been challenging. This report will summarize some universal concepts with regard to the conduct of clinical trials, and will review other issues that specifically pertain to successful clinical trials of therapeutic interventions in dry eye disease.

Methods The Dry Eye Workshop involved the deliberations of many contributors through an iterative process that ultimately produced a harmonized summary of current knowledge of dry eye disease (2007 Report of the International Dry Eye Workshop, Ocul Surf 2007[5(2):63-206). The subcommittee on clinical trial methodology was given the task of performing an evidence-based critical review and summary of the design and implementation of clinical trials in dry eye disease. The group systematically reviewed the medical literature, procedures, and concepts related to clinical trials in general, and considered special issues related to clinical trials involving therapeutic interventions in dry eye disease.

Results The Dry Eye Workshop report makes some specific recommendations for the design and implementation of such trials, based on an assessment of the peculiarities of dry eye disease that complicate clinical trial design, such as the lack of correlation of signs and symptoms, as well as the likelihood of control interventions having a lubricant (placebo) effect. The group also points out potential advantages and disadvantage for conduct of trials in the context of a controlled adverse environment.

Conclusion This report provides a summary of established principles of clinical trial design and implementation and makes suggestions for successful trials of therapeutic interventions in dry eye disease.

Commercial interest
**SIS: A Report from the International Dry Eye Workshop (DEWS)**

* 2315

**Management & therapy**

LEMP MA

**Purpose** The members of the Management and Therapy Subcommittee assessed current dry eye therapies.

**Methods** Each member wrote a succinct level of evidence-based review on an assigned aspect of the topic, and the final report was written after review by and with consensus of all subcommittee members and the entire DEWS membership. In addition to its own review of the literature, the Subcommittee reviewed the Dry Eye Preferred Practice patterns of the American Academy of Ophthalmology and the International Task Force (ITF) Delphi Panel on dry eye disease.

**Results** The Subcommittee favored the approach taken by the ITF, whose recommended treatments were based on level of disease severity.

**Conclusion** The recommendations of the Subcommittee are based on a modification of the ITF severity grading scheme, and suggested treatments were chosen from a menu of therapies for which convincing evidence of therapeutic effect had been presented.

* 2316

**Report on research in dry eye**

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(2) Department of Ophthalmology, Harvard Medical School, Boston, MA

**Purpose** Members of the DEWS research Subcommittee reviewed published research to determine if there is sufficient evidence to define the basic mechanisms underlying dry eye disease.

**Methods** Evidence-based research was evaluated concerning the changes in the tear film, lacrimal gland and accessory lacrimal glands, ocular surface epithelia (including cornea and conjunctiva), meibomian glands, lacrimal duct system and the immune system in dry eye. Consideration was given to both human and animal research data.

**Results** Results are presented as a series of information matrices, identifying what has been learned about dry eye since the previous Dry Eye Workshop, providing supporting references. Areas for further investigation are identified.

**Conclusion** Given insufficient evidence to define the basic mechanisms giving rise to dry eye disease, a hypothesis for the mechanisms underlying the disease is presented.
SIS: Glaucomatous optic neuropathy

* 2331
An overview of the pathogenesis of glaucomatous optic neuropathy

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Glaucoma, clinically, is characterized by degeneration of the optic nerve and progressive visual field loss, associated with elevated intraocular pressure (IOP). The exact cause of glaucomatous optic neuropathy is not well established, although many risk factors have been determined including elevated IOP, family history, race, age, systemic diseases and myopia. Elevated IOP is the most investigated factor among others because it is the main well-known treatable one for glaucoma. The primary components of glaucomatous optic neuropathy are specific changes in the optic nerve head and in the retina. In the optic nerve head, these can be classified as axonal and non-axonal effects (alterations in extracellular matrix and astrocyte). In the retina, retinal ganglion cells appear to undergo morphologic changes before cell death. These changes give a result as an increase in number of retinal ganglion cells and a decrease in visual acuity. Nowadays, lowering IOP alone is not sufficient for the long-term stabilization of visual functions in glaucoma patients. Some abnormalities in optic nerve head and retina may affect relative susceptibility to IOP and explain progressive optic nerve damage and visual field loss, although adequate IOP control. Moreover, optic disc excavation and nerve fiber layer losses of up to 40% have been shown to occur, prior to visual field loss has been determined. In addition to vascular and mechanical theory for glaucomatous optic neuropathy include excitotoxic damage, insufficiency in neuron growth factors, immune-mediated neuron destruction, and oxidative stress. The exact role of these factors and their effects on glaucomatous damage and apoptosis is still controversial.

* 2332
Optic nerve injury models in the rat

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Glaucoma is a degenerative disorder of the optic nerve which can be secondary to many diseases and the pathology occurs at the optic nerve head (ONH). It is important to know the effects of cell types, cell interactions, the trophic factors, receptors, channels, and matrix molecules in glaucoma. Research on the optic nerve in rats, mice, and nonhuman primates are necessary not just in retina and optic nerve but also in central nervous system. Experimentally-induced animal models help in understanding how retinal ganglion cells (RGC) die and how ON take place in this process. Special care must be taken to select controls and to use adequate number of animals. Experiments should be performed to understand the mechanism of pressure-induced optic nerve damage and protect the optic nerve in glaucoma. An effective model should allow for the obstruction of aqueous humor outflow in order to raise IOP and for monitoring of intraocular pressure. Raised intraocular pressure, altered ocular perfusion, loss of auto regulation, alteration of vascular structure and characteristics of the blood brain barrier at the optic disc are disposability candidates in the pathogenesis of glaucoma. Animal studies indicate a response inoxudative metabolism to varied IOP and mean arterial blood pressure, and that low blood pressure leads to loss of autoregulation and ischemia of the optic nerve head. A number of techniques for increasing the intraocular pressure in rat are available. One of them involves sclerosing the trabecular meshwork using hypertonic saline. By cannulating the ciliary body and injecting the corrosive saline retrogradely into the canal of Schlemm, it is possible to scar and close up the trabecular meshwork. Intraocular pressure elevates in approximately a week and lesions begin to develop. The lesions seen in the rat optic nerve following this procedure includes a decrease in axon density and an increase in vascular bodies and vacuoles that is most prominent in the superior region of the optic nerve. We also carried out different models of rat studies either by damaging the ON mechanically or by ischemia to show the histological alterations in the ONH and retina. We also investigated the effects of oxidative stress and apoptosis on these alterations. Understanding the mechanism of nerve damage in these different models may provide new insights into the pathogenesis of human glaucoma.

* 2333
Cell death in optic neuropathy

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ABSTRACT NOT PROVIDED

* 2334
Effects of primary open-angle glaucoma on the globe, cross sectional area and morphology of the optic nerve and volume of the lateral geniculate body: a longitudinal study

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Glaucoma is a group of disorders that lead to damage to the optic nerve, which, in turn, causes vision loss that may progress to blindness. Glaucoma is characterized by increased pressure within the globe. Open angle glaucoma is by far the most common type of glaucoma. In open angle glaucoma, Schlemm channels in the iris-corneal angle gradually narrows with time, impairing the drainage of the aqueous humor. The buildup of aqueous humor causes an increase in the intraocular pressure. This increased pressure pushes on the surface of the optic nerve and the retina, reducing the blood supply to the optic nerve. Twelve patients with primary open-angle glaucoma and twelve age-, sex- and stature-matched healthy controls had high resolution MR imaging of the eye. T1 and T2 weighted MR images were acquired with a 1.5 Tesla imager (GE Medical Systems, Milwaukee, USA) using inversion recovery and fast spin echo sequences. Linear measurements of the distance between the angles formed by iris and ciliary muscle, depth of the anterior chamber, thickness and diameter of the lens, distance between the anterior and posterior poles of the globe were performed. Cross-sectional area of the optic nerve was calculated at 5 mm anterior to the optic chiasm using planimetric methods. The boundaries of the lateral geniculate body were delineated on orthogonal images and its volume was measured by multiplying the slice thickness with the area of each section. MR imaging and measurement protocol was repeated a year later to examine the changes. No significant changes were found in distance between the iris-corneal angles, anterior chamber depth, lens thickness and diameter, and the distance between the anterior and posterior poles of the globe were performed. Cross-sectional area of the optic nerve was calculated at 5 mm anterior to the optic chiasm using planimetric methods. The boundaries of the lateral geniculate body were delineated on orthogonal images and its volume was measured by multiplying the slice thickness with the area of each section. MR imaging and measurement protocol was repeated a year later to examine the changes. No significant changes were found in distance between the iris-corneal angles, anterior chamber depth, lens thickness and diameter, and the distance between the anterior and posterior poles of the globe were performed.
Dissectional and endoscopical anatomy of the lacrimal duct

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Purpose: A better knowledge of the external and internal anatomy of the lacrimal duct is essential for any intervention. The induced pressure of the medial canthal ligament into the lacrimal sac might be considered as one of the reasons of epiphora.

Methods: In this study, cadaver direction, trans-canaliculard and endonasal endoscopy are used to provide a better intuition of anatomy.

Results: Concerning the medial canthal ligament, cadaver study of the lacrimal duct reveals that there are two portions: the posterior portion and anterior one which passes over the lacrimal sac and inserts on the lacrimal bone. The medial portion of the medial canthal ligament is connected by the medial part of the tarsus superior and inferior. There are fascial connections between the anterior part of the ligament and the lacrimal sac and between the anterior part of this ligament to the orbicular superior and inferior muscles. The lacrimal duct starts from the Punctum superius and inferius and terminates in the nose. The inferior opening of the duct comes in the nose on the level of the inferior turbinate. Collaps of the canalculus or the lacrimal sac is investigated by trans-canaliculard endoscopy. It is observed that the pressure induced by medial canthal ligament into the lacrimal sac is the main reason of collaps of the canalculus or the lacrimal sac.

Conclusion: Trans-canaliculard and endonasal endoscopy which are complementary to dissectional anatomy, provide a better insight of the naso-lacrimal duct anatomy and physiology. This knowledge is mandatory for any naso-lacrimal duct interventions. Epiphora with exclusion of the other etiology (allergy, foreign body) than obstruction, can be caused by collaps of the lacrimal sac due to pressure of the medial canthal ligament.

Preoperative asseessment before surgery of the lacrimal pathway

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Methods: Dacryocystorhinostomy (DCR) consists of diverting the lacrimal flow into the nasal cavity through an opening made surgically in a standard fashion at the level of the frontal process of the maxilla. Indication for surgery is low obstruction of the lacrimal pathway resistant to the medical treatment. To make the differential diagnosis between hyperscretion and epiphora, a high or a low obstruction, an anatomical or a functional obstruction of the lacrimal pathway, a preoperative evaluation of all the lacrimal pathway is mandatory. The authors present their experience and comment the technique, the advantages and limits of different investigations such as the ophthalmological examination including Jones tests 1 and 2, and seringing, dacryography, dacryoscan, scintigraphy of the lacrimal pathway and also give some words about the endoscopy of the lacrimal pathway. They also present the results of a survey conducted among the Belgian ophthalmologists confirming that only a minority of them perform a series of investigations (imaging) before a DCR.

Radiologic diagnostic of the lacrimal system. Measurements of the bony lacrimal canal in a European population

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Purpose: To examine the bony anatomical structures and dimensions of the nasal lacrimal duct.

Methods: Patients who underwent a routinely multidose Spiral CT examination of the nasal sinuses were retrospectively reexamined. The original data set with thin slices in 0.75mm was used to reconstruct the nasal lacrimal duct in 3 dimensions (axial, coronal, sagittal).

Results: We found a spectrum of the diameter of the bony canal from 3.0-4.0mm. There was no significant difference in sex and also in the population based on typical swiss names and more foreign names.

Conclusion: Measurements of the bony canal are very important if lacrimal interventions like balloon dilatation or stent implantation is performed. Our results of the bony canal were similar results to Jansen et. al. (2001). This population based study proved no difference in between the races.

External dacryocystorhinostomy

DUCASSE A

Paris

Purpose: Indications, techniques and complications of external DCR.

Methods: Indications are lacrimal stenosis with clinical symptoms: epiphora, dacryostenosis. The external approach is more specifically interesting in case of canicular stenosis associated, when a skin approach is necessary or in case of lacrimal tumor. The endonasal approach is the best way in case of nasal or sinus pathology, failure of DCR or lacrimal abscess and for us in case of bilateral DCR. Technique is always the same, under general or local anesthesia with nasal vasoconstriction. After disinfection, probing, the skin incision is short and rectilinear inside or outside the angular vessels. Marking is the anterior lacrimal crest. After section of the medial canthal tendon, an ostetomy is performed; it must be wide with or without anterior ethmoidectomy. We make two anterior mucosal flaps which are sutured and fixed at the peristome. We do not use systematically a bicanalicular intubation; we put an intubation in case of canicular stenosis, or for a second DCR or in case of surgical difficulties. The main complications are haemorrhages, cribral plate fracture with cerebrospinal leakage, infection, pain and in a few case, a bad scar.

Results: The results are good. Anatomical and functional success are found in 88% to 96%, for us 91.2%. The failures occur during the three first months.

Conclusion: Compare internal or endonasal DCR, the advantages for the external way are cost, rapidity and usual practise. Endonasal DCR needs a lot of material, an apprenticeship and usually a scanner before the surgery and systematically a bicanalicular intubation. In conclusion, external DCR remains an easy technique, which is quick, with high success rate and few complications.
Endoscopic endonasal dacryocystorhinostomy

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Mont Godinne

Methods With the advances of the endonasal endoscopic surgery, DCR performed through the nose becomes with the time a viable and highly effective alternative to the conventional DCR performed through an external approach. The optimal indication is the low obstruction of the lacrimal pathway. This requires a precise preoperative assessment of the lacrimal pathway including a complete ophthalmological examination, sering, and probing of the lacrimal pathway and an imaging (dacrocystography and in some cases, a scintigraphy of the lacrimal pathway). The authors present the technique using the powered instrumentation. They emphasize the importance of the resection of the vertical part of the uncinate process and a large drilling of the frontal process of the maxilla in order to expose widely the nasolacrimal duct and the inferior part of the lacrimal sac. Stenting is not routinely necessary except when the epithelium of the canalicular system is damaged by the probing or when a high obstruction of the lacrimal pathway is associated to the low obstruction or in case of functional obstruction. A review of their results and those published in the worldwide literature is exposed.

Results With good selection of patient the success rate is around 90%.

Non endoscopic endonasal DCR

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Purpose During this lecture we will describe a nonendoscopic endonasal DCR technique.

Methods It is based on the principles and concepts of the endoscopic approach using standard oculoplastic equipment without the need for an endoscope. Surgical loupes and a nasal speculum are used for direct visualization of the osteotomy site. A vitrectomy light pipe through the upper canaliculus into the lacrimal sac is providing transillumination through the thin lacrimal bone within the nose. Formation of the osteotomy is performed using a Kerrison rongeur.

Results Overall success rate better than 90%.

Conclusion Nonendoscopic endonasal DCR is a safe and effective method in treating epiphora with an overall success rate better than 90%.
Antimicrobial peptides on the ocular surface in health and disease
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Purpose Antimicrobial peptides (AMPs) are the eukaryotic analogues of antibiotics and of paramount importance in host defence. We report herein the expression of a spectrum of AMPs at the ocular surface in infective conditions compared to controls.

Methods RNA obtained from impression cytology samples of conjunctival and corneal epithelial cells of normal persons and of patients with bacterial, viral, acanthamoeba and dry eye disease underwent reverse transcription - Polymerase Chain Reaction (PCR) to assess expression of the AMPs HBD-1 to -4, (Human Beta Defensin), Defensin, LL-37 (or Cathelicidin), LEAP-1 and LEAP-2 (Liver Expressed Antimicrobial Peptide) and a number of putative AMPs.

Results HBD-1 and -2 were universally expressed in both health and disease. HBD-3 was seen only occasionally in infection and not in controls and seemed to be commonly expressed in dry eye disease. LL-37 occurred in both normals and infective groups but the level of expression was variable and was less in acanthamoeba infection. Likewise LEAP-1 and -2 were commonly seen both in control and infection and appeared to be more strongly expressed in the former. The latter AMPs, i.e. the putative AMPs were not expressed.

Conclusion AMPs have a distinct profile on the ocular surface as opposed to other mucosal surfaces and different disease conditions appear to further modify gene expression. In contrast to other surfaces HBD-1 and -2 both appear to be constitutively expressed on the ocular surface and other AMPs are more strongly expressed in certain disease states than others whereas others are more common in control groups than in infective conditions.

Gene expression profile of Limbal Epithelial Stem Cell niche
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Purpose It is established by laboratory and clinical evidence that corneal stem cells are localised at limbus but are not isolated as yet due to the lack of stem cell markers. Recently we had discovered a novel anatomical structure at limbus, termed as the Limbal epithelial crypt (LEC), which have the potential of a stem cell niche as determined from previous anatomical and immunohistological studies. We aim to characterise the differential gene expression of the LEC by comparing with other regions of the ocular surface epithelium by 30,000 genes human oligonucleotide microarray analysis with the view to find putative stem cell marker.

Methods On the frozen cryostat sections of the corneoscleral epithelium from cadaver donor tissue; Laser Capture Microdissection (LCM) by PALM® Microbeam systems was done. From the LMD tissue the RNA was extracted, amplified and hybridised to human oligonucleotide array chip. Raw data was obtained with Genepix Pro 6 software and uploaded on BASE where filtration and normalisation was done. Further analysis was done on T-Mev software by SAM and T Test.

Results Microarray analysis was performed on BASE and with Significance Analysis of Microarray (SAM) on T-Mev. The analysis showed significant gene expression indicative of stem cells such as specific and differentiation markers,Transcription factors, cell adhesion molecules, genes involved in cell metabolism and respiration showed up regulation in the LEC.

Conclusion The microarray analysis data supports our hypothesis that LEC harbours stem cells along with neighbouring niche cells and hence represents a Limbal Stem Cell niche: This is being confirmed with real time PCR.

Characterization within and around the Limbal Epithelial Crypt
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Purpose The Limbal Epithelial Crypt (LEC) is an anatomical structure that is found between the junction of the cornea and sclera and is in a unique position to make it an ideal structure to examine further. Previous studies have demonstrated the LEC to have properties that suggest it may be a stem cell niche. Basal cells of the LEC are significantly smaller than basal cells found in adjacent rete pegs, and morphologically they have a higher nuclear:cytoplasmic ratio. We set out to examine LEC further by exploring the surrounding LEC matrix proteins, and with known differentiation markers.

Methods Donated corneo-sclerio rims were cut into eight equal sized pieces and frozen. Each piece was cut into 7µm serial sections, and was examined by microscopy for LEC structures. Identified LEC was collected on slides and stored until they were fixed in acetone and processed by standard immunofluorescence techniques for each differentiation marker.

Results Tenascin C was more positively taken up by the basement membrane of the LEC compared with the surrounding limbus. In addition, staining for desmogelin was negative against isolated small subpopulations of cells within the basal regions of the LEC.

Conclusion The LEC structure demonstrates properties that may identify this as a possible stem cell niche. Further studies are necessary to determine the significance of the LEC in its role in stem cell maintenance.

Cytokeratin 8 and 18 expression in human corneal endothelium
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Purpose To determine if cytokeratins (CK) 8 and 18 are expressed in healthy corneal endothelium.

Methods Five healthy human corneal discs not suitable for transplantation were used. The mean age of the corneal donors was 70 years (range: 51-83 years). The corneal buttons were dissected into two parts. One half was used for expression cytology of the endothelium followed by semi quantitative RT-PCR and immunohistochemical detection of CKs. The second half was frozen and cryosections were prepared. Impression specimens as well as cryosections were fixed and indirect immunofluorescence staining was performed using antibodies against CK8, CK18 (Chemicon) and CK8/18 (Dako). The percentage of positive cells and the intensity of the staining were assessed using fluorescent microscopy. A minimum of two hundred cells were counted per specimen.

Results All impression specimens displayed cells with the hexagonal morphology typical of the corneal endothelium. The mean number of positive cells was 37% for CK8, 8% for CK18 and 42% for CK8/18. The mean number of positive cells in cryosections was 47% and 38% for CK8, CK18 and CK8/18, respectively. Furthermore, expression of the CK8 and CK18 genes was present in all corneas.

Conclusion We detected both CK8 and CK18 in the endothelium of all specimens at both protein and mRNA levels. These results unambiguously demonstrate that cells of the corneal endothelium express the CK pair 8/18 and share some features with simple epithelium.
**2355**

**Difference of ocular injury by millimeter wave exposure with different frequency**

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**Purpose**
The validity of the safety standard (10-300 GHz 10 W/m² in public, JCNIRP) of a wide-ranging quasi- and millimeter wave was examined from ocular temperature changes during exposure and ocular injury after exposure.

**Methods**

- Pigmented rabbits were exposed unilaterally to 18, 22, 26.5, 35, and 40 GHz quasi- and millimeter wave with a lens antenna for 3 minutes. The focused beam was exposed to the center of the rabbit cornea. Ocular temperature changes (cornea, lens, vitreous, retrobulbar) during a 3 min. exposure were measured with a fluoroptic thermometer and corneal surface temperature was measured with a thermography camera. For evaluating ocular injury, a rabbit was exposed to one frequency (18, 22, 26.5, 35, 40, 60 GHz) with 800, 1500, 3000 mW/cm² for 6 min. Ocular changes were evaluated by slit lamp and ocular inflammation was measured by laser flare meter immediately after and 1 day after exposure. Enucleated lens was observed with lens epithelial cell flat mounting.

**Results**

- Cornea and lens temperature rise were detected by exposure with all examined frequencies. The highest ocular and corneal surface temperature was 40 GHz, followed by 35, 22, and 18 (almost the same), and the lowest was 26.5 GHz. Ocular injury by 800 mW/cm² for 6 min. exposure was seen only with 40 GHz. Vitreus and iris vasodilation were seen immediately after exposure, and corneal epithelial defect at the center of the cornea and corneal opacity were seen one day after exposure. 40 GHz 3000 mW/cm² for 6 min. exposure developed cornea and lens opacity. But the same dose of 60 GHz exposure showed only corneal opacity.

**Conclusion**

It was suggested that thermal and biological reactions differed according to frequency.

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**2356**

**Remodelling of collagen fibrils and proteoglycans in the zebrafish cornea during development**

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**Purpose**

Collagen fibrils and proteoglycans are the main components of the corneal extracellular matrix and corneal transparency depends crucially on their proper organisation. We investigated their formation and arrangement in the developing cornea of the zebrafish, a major model of vertebrate development and genetic disease.

**Methods**

- We employed thin-section electron microscopy to investigate the ultrastructure of the zebrafish cornea at different stages of development.

**Results**

- Layering of the zebrafish cornea into an epithelium, Bowman layer, stroma and endothelium was observed by 72 hours post-fertilization. At this stage, the stroma contained orthogonally arranged collagen fibrils and small proteoglycans. The density of proteoglycans increased gradually throughout subsequent development. In the stroma of 2 week old larvae, the collagen fibrils were organized into thin lamellae for the first time and were separated by very large, randomly distributed proteoglycans. At 4 weeks, a regular arrangement of proteoglycans around the collagen fibrils was observed for the first time and the lamellae also thickened.

**Conclusion**

This is the first report of collagen fibril and proteoglycan development in the zebrafish cornea and it directly correlates collagen fibril and proteoglycan organisation of the zebrafish cornea with that of the human cornea. The similarities between the two species, including the possession of a Bowman layer, suggest that the zebrafish could serve as a model for the genetics of human corneal development and inherited disease.
【2361】
Predicting the prognosis of ciliochoroidal uveal melanoma by its clinical characteristics: a European ophthalmic oncology group collaborative study

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Purpose To assess clinical characteristics independently associated with mortality from uveal melanoma using a large contemporary European dataset in order to improve future classifications.

Methods Data of 8340 patients who had a choroidal and ciliary body melanoma managed by one of five participating oculary oncology centers directed by members of the European Ophthalmic Oncology Group (EOOG) were collated. Variables requested included date of treatment and last follow-up, survival status, largest basal tumour diameter (LBD), tumour thickness, ciliary body involvement and extracoroidal extension. Data were analysed with the product-limit method and Cox regression.

Results Valid LBD and thickness was available for 7922 and 8111 melanomas, and ciliary and extracoroidal extension for 7418 and 6087 tumours, respectively. Complete data for all 4 variables were available for 5469 tumours (66%). Mean follow-up time was 4.0 years (interquartile range, 1.5-7.3). In Cox regression, all variables were significantly (P<0.0001) and independently associated with melanoma related mortality. Covariate-adjusted hazard ratio (HR) was 1.15 for LBD and 1.06 for thickness (each 1 mm increase), and 1.72 for ciliary body involvement and 2.29 for presence of extracoroidal extension (yes or no).

Conclusion Choroidal and ciliary body melanomas may effectively be categorised by LBD and thickness, and subclassified by presence of ciliary body and extracoroidal extension. All four variables are recommended to be included in future clinical classifications of uveal melanoma. How ciliary body and extracoroidal extension could be collaboratively coded in more detail remains to be discussed.

【2362】
Fish analysis of chromosome 3 and 6 on fine needle aspiration biopsy identifies distinct uveal melanoma subgroups

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Purpose Some evidence suggests that uveal melanomas develop following one of two ways in a pathogenetic model, one starting with a loss of chromosome 3 and the other with extra copies of 6p (6q), having different prognostic outcome. The aim of this study was to analyze the role of Fluorescence In Situ Hybridization (FISH) applied on Fine Needle Aspiration Biopsy (FNAB) specimen to in vivo verify the presence/absence of this bifurcated pathogenetic and prognostic model.

Methods Thirty-five consecutive patients, affected by posterior uveal melanoma (≥3.5 mm in thickness), scheduled for iodine-125 brachytherapy, underwent intraoperative trans-scleral FNAB just before plaque implantation. Specimens underwent Fluorescent in Situ Hybridisation (FISH) to detect chromosome 3 and 6 (6q 6p) status.

Results Minimum follow-up was 12 months (range: 1.2-40 months). All cases gave enough material for cytogenetic analysis. 6p and monosomy 3 were mutually exclusive in 29 cases (83%). Three cases (8.5%) showed no chromosome 3 and 6 abnormalities and three cases (8.5%) showed both monosomy 3 and 6p. Monosomy 3 was not significantly related (p=0.5%) to tumour dimensions and tumour location (choroidal-ciliary body). No major complications or extracoroidal extension were documented. Three patients (8.5%) with monosomy 3 developed metastatic disease during the follow up.

Conclusion In-vivo detection of cytogenetic prognostic-related characteristics in posterior uveal melanoma is possible analyzing cytologic specimens obtained by transscleral FNAB before plaque implantation. FISH analysis for chromosome 3 and 6 status could offer more prognostic information than monosomy 3 alone.

【2363】
Treatment of juxtapapillary choroidal melanoma

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Purpose To compare different treatment for juxtapapillary choroidal melanomas.

Methods Retrospective study comparing recurrence, survival and complications in 14 patients treated with proton beam treatment. 11 patients treated with stereotactic radiotherapy and 28 patients treated with Ruthenium brachytherapy in combination with transscleral thermotherapy.

Results Mean follow-up time was 45.3 months, in which 4 patients died due to their melanoma. There was a significant faster decrease of the tumour prominence in the Ruthenium group. Earlier radiation retinopathy (6 months onwards) in the Ruthenium group, but no significant more radiation retinopathy after 5 years. The vision after 5 years was significant lower in the stereotactic radiotherapy group, but this was also due to the fact that there were more patients with a juxtapapillary melanoma on the macular side in this group.

Conclusion A prospective randomised study is needed to determine which therapy is best in treating juxtapapillary melanomas. In this retrospective study, there was no difference in local recurrence or survival in the 3 treatment groups.

【2364】
Matrix metalloproteinase-2 (MMP-2) is expressed in melanoma associated spongiform scleropathy (MASS)

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Purpose To correlate the expression of matrix metalloproteinase 2 (MMP-2) with the occurrence of melanoma associated spongiform scleropathy (MASS) and tumour extension in eyes with uveal melanoma.

Methods Twenty-two specimens of human eyes with choroidal melanoma were selected for this study. Eleven were MASS positive with tumour extension and eleven were MASS negative without tumour extension. Sections were examined for MMP-2 expression by in situ hybridization. Immunohistochemical study of the same specimens was conducted. For double labelling, primary MMP-2 antibodies directed against fibroblasts and macrophage were applied.

Results Distinct hybridization signals with MMP-2 were seen in fibroblast-like cells located in the sclera in 14 out of 22 melanoma eyes. MMP-2 mRNA expression was detected in ten eyes with MASS and tumour extension. In eight the signals were seen in numerous cells. In melanoma cases without MASS, MMP-2 signals were detected in four cases and only one case showed numerous positive cells. The expression of MMP-2 was further demonstrated at protein level by immunostaining, confirmed by double labelling experiment. Tumour macrophages were also found to harbour MMP-2.

Conclusion MASS which is considered as scleral degradation process, may be mediated by MMP-2 produced by scleral fibroblasts under the influence of tumour cells and/or tumour macrophages. This is manifested by the significantly higher expression of MMP-2 in MASS areas indicating that MMP-2 is involved in the development of MASS. MASS changes may represent a step in the invasion of uveal melanoma. This association between MASS and MMP-2 could be considered as a new prognostic marker in uveal melanoma.
Spontaneous choroidal haematomas: differential diagnosis with uveal melanoma: a retrospective review of 95 cases

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Purpose Introduction: Patients with peripheral choroidal melanoma are often misdiagnosed as uveal melanoma

Methods We have reviewed the clinical, MRI and ultrasonographic characteristics of patients sent to Curie institute for suspected uveal melanoma between 1998 and 2006 and of whom final diagnosis was choroidal hematoma

Results 95 patients were seen with this diagnosis (for 2357 patients treated for uveal melanoma during the same period); the age varies between 54 and 92 years with a median age of 77 years; there was a history of macular degeneration in 27 cases and posterior pole drows in 3 cases. 11 patients were taking anticoagulant or antiaggregant medications. There was sudden loss of vision in 35 cases and a vitreous haemorrhage in 18 cases. The lesion was located in the posterior pole in 28 cases and in the periphery in 67 cases. The thickness measured by B-scan ultrasonography varied between 1 mm and 7, 8 mm with a mean thickness of 2, 86 mm. MRI was necessary in 27 cases and color doppler ultrasonography in 10 cases. These exams usually shows peripheral enhancement with gadolinium (compared to full thickness gadolinium enhancement in melanomas) and no vessels with doppler (compared to visible vascularisation in melanomas). A follow-up was performed in 42 patients showing progressive fibrin scarring

Conclusion These peripheral hematomas can be due to peripheral exudative chooroiritis or to ruptured retinal maculopathy, they are more frequent in elderly patients with vascular diseases. The diagnosis of choroidal hematomas is generally made by fundus examination but MRI and color doppler imaging can be useful

Bilateral retinoblastoma: successful conservative treatment of both eyes

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Purpose Conservative treatment (focal therapy, local and systemic chemotherapy, radiotherapy) is today the standard in Retinoblastoma treatment. Preservation of both eyes is unfortunately, not always, possible.

Methods From 1959 to 2007, 442 cases of retinoblastoma have been diagnosed and treated at the Retinoblastoma Referral Center of the University of Siena. 175 cases were bilateral.

Results 25 out of 175 bilateral cases have been conservatively treated. Age at diagnosis, sex, hereditary, stage at diagnosis (Reese Classification and ABC Classification), remission patterns and therapeutic protocol are reported. The age at diagnosis ranged from 4 days to 15 months (mean age: 5.4 months). 8 out of 25 were females, 17 out 25 were males. 13 out of 25 were familial cases. Stage at diagnosis (50 eyes) was I in 16 eyes, II in 12 eyes, III in 8 eyes, IV in 11 eyes. V in 3 eyes. 25 out of 25 received chemotherapy and focal therapy according to the Italian protocol. 12 out of 25 relapsed. 9 out of 12 received new cycles of focal and chemotherapy. 2 out of 12 received also brachytherapy. In 2 out of 12 subconjunctival carboplatin has been used. In only 1 case (familial case) external radiotherapy was requested and the child died for a second tumor one year later. 5 out of 25 developed new foci; immediately treated only with focal therapy.

Conclusion The prognostic factors for successful conservative treatment are evaluated.
**Free Papers: Keratoconus and corneal grafting**

**2411**

First experience with corneal collagen UV cross-linking treatment in keratoconus patients

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**Purpose**

Corneal collagen cross-linking by UVA: riboflavin represents a new method for the treatment of progressive keratoconus.

**Methods**

In the presentation we will present our first 10 cases of keratoconic eyes (maximum K value, 45–51 diopters) with the follow up time from 4 to 7 months. A complete ophthalmologic examination with uncorrected visual acuity (UCVA) and best corrected visual acuity (BCVA) were performed. Listed examinations were performed before surgery and at 1, 2, 3 and 7 months after surgery: corneal computerized topographic examination, pentaacam examination, endothelial cell count and intraocular pressure (IOP) evaluation.

**Results**

Comparative preoperative and postoperative results showed increases of 1.4 lines for UCVA (P = 0.00021) and 1.66 lines for BCVA (P = 0.00051). Topographic analysis showed a mean K reduction of 1.9 ± 0.15 diopters (D) in the central 3.0 mm. Statistical analysis of IOP, corneal thickness and endothelial cell count did not show significant differences. Corneal and lens transparency remained unchanged. Topographic analysis findings of corneal symmetry showed a trend toward increasing corneal symmetry with a major reduction in asymmetry between vertical hemiretinas.

**Conclusion**

Refractive results showed a reduction of about 1.5 D in the mean spherical equivalent, topographically confirmed by the reduction in mean K. Results of surface topographic analysis showed improvement in morphologic symmetry. Long-term results are necessary to evaluate the duration of the stiffening effect and to exclude long-term side-effects.

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**2412**

Wedge resection for high astigmatism in penetrating keratoplasty for keratoconus: refractive and histopathologic changes

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**Purpose**

To analyze the refractive, topographic, keratometric and histopathologic changes in wedge resection to correct high astigmatism in penetrating keratoplasty for keratoconus.

**Methods**

A retrospective study was done analysing the following parameters preoperatively, at 1 and 3 years post-operatively: UCVA, BCVA, spherical equivalent, refractive, topographic and keratometric cylinder. We also studied the index of efficacy and safety, as well as the histopathologic results of tissues submitted for analysis.

**Results**

A total of 22 eyes of 21 patients who underwent PKP for advanced keratoconus and wedge resection for high astigmatism 18 years following PKP were included in the study. Mean follow-up time was 3.2 years. All refractive, topographic and keratometric data showed the lowest degree of astigmatism at three years post-op with a tendency towards regression towards five years post-op. Safety index was 1.6 while efficacy index was 0.4. The corrected refractive cylinder at 3 years follow-up was 55.2% while the corrected topographic cylinder was 53% at 3 years. All histopathologic sections of resected tissue were consistent with keratoconus.

**Conclusion**

Wedge resection is safe and moderately efficacious in the correction of high astigmatism post PKP for keratoconus. As previously published by the same authors, our study corroborates that there is a trend towards regression of the desired cylindrical correction thru time. Keratoconus may be a disease that affects the entire cornea and surgical resection does not treat the disease.

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**2413**

Failure of topical Cyclosporine A 2% in patients with severe ocular rosacea after penetrating keratoplasty

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**Purpose**

To evaluate cyclosporine A (CsA) 2% eye drops in the prevention of acute rejection after penetrating keratoplasty (PKP) in patients with severe ocular rosacea.

**Methods**

IRB approval (Poitiers School of Medicine) was given an Informed Consent was obtained from all patients according to French Regulations, 35 patients with severe Rosacea that underwent a PKP from 2000 to 2003 for pseudophakic bullous keratopathy were topically treated with CsA. The patient’s charts, the level of rejection risk, the IOP and the graft outcome were analyzed.

**Results**

No systemic effect was detected. 21 rejections developed after a median of 18.5 months. Ten rejections under CsA treatment were reversible.

**Conclusion**

CsA 2% eye drops failed in being an effective treatment in the prevention of acute rejection in patients with severe ocular rosacea.

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**2414**

Refractive and biomechanical changes with intrastral ring segments for keratoconus

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**Purpose**

To analyse the refractive changes by Power Vector Analysis and the changes in corneal elasticity with the Ocular Response Analyzer (ORA).

**Methods**

Refraction data at one day and three months post-op was available in 30 eyes (30 patients) and biomechanical data in 10 eyes (10 patients). Visual acuity and topography was analysed in all patients. The type of intrastral ring segments used were 85% Ferrara and 15% Intacs. Spherical equivalent M, the astigmatism components J0 and J45, and the length of the Power Vector are calculated.

**Results**

The mean length of the Power Vector changed from 6.1 D (+/- 1.0 confidence interval [CI]) pre-op to 3.1 D (+/- 0.6 CI) at one day post-op and was 3.5 D (+/- 0.9 CI) at three months post-op. ORAs Corneal Hysteresis Factor in keratoconus patients (mean 7.4 +/- 0.5 CI) was significantly lower than in controls (mean 11.2 +/- 0.8 CI) but did not change after intrastral ring implantation.

**Conclusion**

Intrastral ring segments for keratoconus reduced the spherical equivalent refraction and the amount of astigmatism at one day and three month post-op, with some regression between 1 day and 3 month post-op. Corneal hysteresis appeared not to be altered by the intrastral ring segments.
Orbscan evaluation of keratoconus

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Purpose To evaluate corneal structure in keratoconus eyes using "in vivo" laser confocal microscopy.

Methods In this prospective study we evaluated 23 eyes of 12 consecutive patients with keratoconus (6 men and 6 women, mean aged 37.30 ± 9.93; range: 23-52 years). 23 eyes of normal patient were the control group. Keratoconus were classified in mild (-47D), moderate (-47-55D) and severe (-55D). We evaluated using laser confocal microscopy the corneal epithelium, Bowman layer and anterior, intermediate and posterior stroma. We quantified the mean density per mm2 of basal epithelial cells, anterior stromal keratocytes (50 µm down Bowman layer), intermediate and posterior (50 µm up endothelial cells). All measurements were done by the same masked examiner.

Results Of the 23 eyes, 16 had mild keratoconus, 5 moderate keratoconus and 2 severe keratoconus. Basal epithelial cells density was 5600.22 cell/mm2 ± 975.13. Keratocyte density was 391.04 cell/mm2 ± 105.39 in anterior stroma, 353.83 cell/mm2 ± 53.28 in intermediate stroma and 349.43 cell/mm2 ± 61.34 in posterior stroma. We found a reduction of cellular density in all measurements with a p<0.01 when compared with normal population.

Conclusion Using laser confocal microscopy HRT2-BCM is possible to measure the reduction of the keratocyte population in keratoconus eyes. The clinical relevance and implications that this fact may have need further research.

A software system for automatic estimation of corneal epithelial cell density in confocal microscopy

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Purpose The problem of reliable automatic estimation of density of corneal epithelial cells in images from confocal microscopy was addressed. The reliability of estimated densities should be comparable to that of manual cell count.

Methods The spatial frequencies contained in epithelial images can be extracted with suitable mathematical techniques (2-dimension Discrete Fourier Transform, DFT). An algorithm for reliably identifying the spatial frequency information and for deriving from it an estimate of the cell density has been developed. A preliminary entropy-based pre-processing was carried out to extract from the whole image the ROI (region of interest) where cells are visible. A prototype of the whole algorithm was implemented in the Matlab language and run on a personal computer. A preliminary evaluation was performed on a data set containing 24 images from normal subjects (see e.g. enclosed image), acquired with a Confoscan 3 confocal microscope (Nidek Technologies, Italy). Reference manual counts were performed on each image by an experienced ophthalmologist.

Results Mean percent difference of automatic densities vs. manual ones was 1%, with std dev 1.8% and range [17% - 18%]. Mean percent absolute difference was 9%, with std dev 4% and range [1% - 18%]. Running times of the prototype were in the order of 40 seconds per image.

Conclusion A new algorithm was developed for the automatic estimation of epithelial cell density. A preliminary evaluation of the proposed technique confirmed its capability of reliably estimating corneal epithelial cell density in confocal images of normal subjects. Implementation of the algorithm with a more efficient computer language, e.g. C++, will allow execution times in the order of 2-3 seconds.

Commercial interest

Corneal graft endothelial viability assessment using the triple labeling Hoechst/Ethidium homodimer/Calcein-AM: technical improvements using 3D microscopy

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Purpose A reliable viability test for corneal endothelium is necessary for the assessment of new processes likely to affect it (storage media, lamellar cutting methods...). Aim: to improve the classical calcin-AM (C)/Ethidium homodimer (E) double staining (live/ dead assay) by adding Hoechst and by using a 3D microscope and image analysis that allow a quantitative assessment on the whole endothelium. As an example of application, the assessment of the actual endothelial viability immediately before graft is given.

Methods The endothelial side was incubated 45 min at 31°C with 200 µl of C(2µM), E (5µM), H (10µM). After flat mount, images of the whole endothelial area (81mm²) were taken using a microscope equipped with a motorized stage (IX71, Olympus) and a x4 objective. Z stacks were taken and an extended focal imaging algorithm allowed taking account of corneal folds. Endothelial cell density (ECD) and mortality were determined by automatic counting of H+ and E+ nuclei. Area of C+ cells was measured.

Results This upgraded staining on the whole endothelium allowed the exhaustive assessment of ECD, cell mortality and metabolic activity. On corneas immediately before graft, it highlighted a dramatic lower number of viable cells than expected with the routine assessment of ECD only, done 48h before graft in the eye bank.

Conclusion The triple HEC staining takes account of the heterogenous pattern of lesions within the whole endothelial layer and is therefore far less biased than assessment done on small cell samples. Its use improves the assessment of all new processes likely to affect the endothelium ex vivo.
A mutation in a novel connexin-like gene affects early lens development in the mouse

**Purpose** Aim of the study was the morphological and genetic characterization of Aey12, a novel dominant mouse mutant suffering from microphthalmia, lens and corneal opacities.

**Methods** A genome-wide linkage analysis was performed using micro-satellite markers. The Aey12 mutation was identified by sequence analysis of positional candidate genes. Histological analysis and in-situ hybridizations were performed at the stages E10.5 - E17.5.

**Results** Aey12 (abnormality of the eye) is a novel dominant mouse mutant, which was recovered in an ENu mutagenesis program at the GSF. Aey12 animals are characterized by small eyes with cornea opacities and lenses with cataracts and vacuoles. Both, heterozygotes and homozygotes, are viable and fully fertile. As major histological defect, the elongation of the primary lens fibers is blocked resulting in the stop of lens development at this stage. Genome-wide linkage analysis mapped the Aey12 mutation on mouse chromosome 10 between the markers D10M1206 and D10M1619. Among the positional candidate genes, one EST (D2300448M01Rik) encodes a connexin-like protein, which is similar to Connexin 43.4. A G-T point mutation was identified at cDNA position 96 resulting in an R12Q amino acid exchange in a transmembrane domain. D2300448M01Rik is expressed in the posterior part of the lens vesicle exactly at the position where the primary fiber elongation starts. Furthermore, an additional expression was observed in many other tissues like brain, lung, heart, liver and kidney.

**Conclusion** The new mouse mutant Aey12 is characterized by an early block of the eye development caused by a point mutation in a novel connexin-like gene.

**Free Papers: Genetics and animal models**

**Reese H.**

**Purpose** Sighted C3H mice with a normal Pde6b gene, were compared to wild-type C3H/HeJ mice with the strain-specific Pde6b(-/-) mutation causing retinal degeneration, BALB/c and C57BL/6 mice.

**Methods** All mice were screened for morphological and visual alterations by funduscopy, slit lamp biomicroscopy, laser interferencemetry, ERG and optokinetic drum.

**Results** In ‘sighted’ C3H mice the mutated Pde6b(-/-) allele of C3H/HeJ mice was replaced by the normal Pde6 allele from BALB/c mice. The presence of the normal Pde6b gene was verified by PCR analysis. Slit lamp biomicroscopy revealed no abnormalities in the anterior segments of the eye in all mice. In the fundus of C3H/HeJ (Pde6b(-/-)) mice the typical pigment patches and vessel attenuation caused by the retinal degeneration were observed, while the ‘sighted’ C3H mice showed a normal fundus appearance, comparable to C57BL/6 mice. The albino BALB/c mice had a red-shining fundus, due to the lack of pigmentation. Scotopic ERG was performed with our screening setup for mice, where BALB/c and C57BL/6 mice showed good responses with well developed a- and b-waves, while C3H/HeJ (Pde6b(-/-)) mice showed no response. Unexpectedly, all ‘sighted’ C3H (Pde6b(+/-)) mice did not show any b-wave response in the scotopic ERG, but scored at least once within 6 trials in the optokinetic drum. As expected C3H/HeJ (Pde6b(-/-)) mice did not respond to the moving stripes of the optokinetic drum, as did the BALB/c mice. A good response to the rotating drum was obtained from C57BL/6 mice.

**Conclusion** ‘Sighted’ C3H mice have a normal Pde6b gene and responded to the rotating stripes in the optokinetic drum, but do not have normal vision due to the lack of the b-wave in the ERG. This work is supported by the NGFN.

**2424**

**Genotype/phenotype features of the Slovenian patients with Stargardt disease: four novel mutations in ABCA4 gene and three novel complex alleles identified**

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**Purpose** To evaluate efficiency of recently developed genotyping microarray, the ABCR4000 chip, in combination with the denaturing high performance liquid chromatography (DHPLC) method sequencing to search for known ABCA4 mutations and to associate the findings with phenotype.

**Methods** 36 Slovenian patients with phenotype of STGD1. were analyzed for a particular mutation-specific sequence on the variations in the ABCA4 gene. In the first step we used the ABCR4000 microarray to identify the known mutations. Patients with one or no mutation on ABCR4000 microarray were subsequently screened by DHPLC and sequencing. Phenotype was assessed by autofluorescence imaging, psychophysical methods and electrophysiology.

**Results** By ABCR4000 microarray, 25 potential disease-associated alleles were identified resulting in a detection rate of 69.4%. With DHPLC, additional 8 mutations were found: four novel were ABCA4 mutations (two frameshift mutations 5977delT, 5716insG and two missense P597S and P640S). Three novel complex alleles were also identified. Mutation in both alleles were found in 9 patients, and in one allele in 14 cases. In the remaining 3 patients no mutations were found. Different phenotypes were characterized by different patterns of autofluorescence imaging, Electrophysiology in general, showed normal flash ERG with abnormal PERG and multifocal ERG.

**Conclusion** Combined approach using ABCR4000 chip and DHPLC/sequencing was efficient in detecting over 90% of potential disease-causing mutations in Slovenian Stargardt patients. 4 of which were novel and show distinct genotype/phenotype patterns.
* **2425**

Mapping genomic loci related to variation in visual quantitative traits in an isolated island community of Croatia

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**Purpose**

It has been hypothesized that isolated human communities are particularly favourable for identifying genetic variants underlying complex human traits and diseases because of their reduced genetic and environmental diversity. The aim of this study was to identify genomic loci that may control variation in visual quantitative traits in 1,057 examinees in isolated island of Vis, Croatia, by positional cloning.

**Methods**

Using refractometer, ultrasound and Canon CR-1DI non-mydriatic fundus retinal camera we obtained a number of visual quantitative and qualitative traits in our set of examinees. All measurements have been adjusted for potential confounders that were identified through an appropriate questionnaire. Both linkage analysis and and genome-wide association approach were used (PLINK and GENABEL softwares) to identify genomic loci that may have significant contribution to the variation observed in those traits.

**Results**

A number of loci have been identified in the genome that approached formal significance levels after Bonferroni correction of 0.6. Those loci now require repeat in other populations to eliminate false positive findings, and further study in bioinformatics and functional genomics to understand their potential role in trait variation and possible development of pathologic states.

**Conclusion**

This study shows that mapping genetic variants underlying complex human traits in isolated population is a feasible and powerful approach. We aim to expand our studies to other Croatian islands to further increase the power of the study.

* **2426**

Autosomal dominant recurrent erosions – from non-existing to being everywhere

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**Purpose**

To describe phenotypes of autosomal dominantly inherited corneal dystrophies characterized by recurrent corneal erosions in Sweden.

**Methods**

We have presented data about families with autosomal dominantly inherited recurrent erosions at national meetings in Sweden for 5 years and urged colleagues to report their existence to us. Their reports have led us to investigate several families. Interviews and clinical examinations were made to establish a pedigree and the specific phenotype for each family.

**Results**

Five families with more than fifty members have been found. The largest was a seven-generation family with 342 members. Another 12 smaller families have been identified. Altogether 870 family members of whom 262 were affected were included. The families originated from all parts of Sweden and one also included members in Finland. The phenotypes vary. For example, the onset were usually at the age of 4-6 years, but in one family before 12 months of age. Also corneal opacifications of varying type and degree were found. In one family opacifications were first noted at the age of about 7 years, but usually first seen at the age of 20-40 years. Vitamin B complex treatment was effectively employed to reduce symptoms and number of recurrences in a family with 175 members. In another family phototherapeutic keratectomy (PTK) cured the recurrent erosions. In yet other families neither vitamin B supplementation nor PTK has been the cure when tried.

**Conclusion**

It seems that inherited recurrent erosions are more common in Sweden than was first believed. Phenotypically it appears to be several different corneal diseases. The ongoing molecular genetic work-up will disclose if several genes are involved and/or several mutations in one or more genes.
Joint Meeting: CONCORDIA: Promoting European Education in Ophthalmology

* 2431
EBO promoting European Network Education
TASSIGNON MJ
EBO, President
ABSTRACT NOT PROVIDED

* 2432
EUPO: teaching the European residents in ophthalmology: history, 2007 and future
SPIEERS W
EUPO, Secretary General
ABSTRACT NOT PROVIDED

* 2433
SOE and the young ophthalmologist
SEREGARD S
SOE, Secretary General
ABSTRACT NOT PROVIDED

* 2434
EVER: Research in ophthalmology and vision in Europe - Visions for the future
PLEVERLI
EVER, President 2007
ABSTRACT NOT PROVIDED
* 2435

Increasing mobility in Europe with SOE and EBO grants

HAWLINA M
EBO, President Elect

ABSTRACT NOT PROVIDED
2441
Nasolacrimal duct interventions - dacryocystoplasty
(Balloon catheter dilatation in pre- and postnasal obstructions)
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Purpose To examine the clinical outcome of the balloon dilatation in stenosis and obstruction of the nasolacrimal duct.

Methods Patients with epiphora and proven stenosis or radiologic short-distance occlusions were successful treated with balloon dilatation alone, of the nasolacrimal duct were treated with balloon dilatation. The diagnosis was established by dacryocystography. The intervention with balloon dilatation was followed by a one-week period of additional treatment with antiinflammatory and antigen(arguments) eye-drops. Patients were followed for clinical evaluation at 6 and 12 weeks and yearly thereafter.

Results Technical success was obtained in 90% of cases with stenosis and in 100% of cases in occlusions. Over a mean follow-up of 6 months patency of the nasolacrimal duct system was achieved in 83 % in stenosis and 66 % in radiologic short-distance occlusions.

Conclusion Balloon dacryocystoplasty is a minimal-invasive alternative, performed in local anesthesia that recover the normal anatomy of the nasolacrimal duct system. It is a good alternative in the treatment of epiphora caused by nasolacrimal duct obstructions.

2442
Endoscopic dacryocystorhinostomy and balloon-dacryocystorhinostomy and transcanicular DCR
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Darmstadt

With the development of miniaturisation of instruments endoscopic techniques got more and more important in ophthalmology. In this abstract endocanicular endoscopic techniques will be presented. The significance of an endoscopic diagnostics will be shown. Developments in the ongoing development in endonasal surgery made it possible that the success rate in internal endonasal Dacryocystorhinostomy is similar to those of an external DCR. With the possibilities of endocanicular endoscopy of the lacrimal duct and the options in surgery which result from that even more options opened up. Those minimised the surgical effort and the postoperative rehabilitation, judging the actual situation of the mucosa endoscopically regarding the position and degree of the stenoses there are different possibilities in rechanneling. With stenoses of the canaliculus or the lacrimal sac there is the possibility using the Erbium-YAG-Laser to do dacryocystoplasty. With subtotall stenoses which show clinically as total stenoses the rechanneling with a miniaturised dilatation is indicated, a Microdiod dacryocystoplasty. Both methods have a success rate of 78-80%.

2443
Combined surgery: endoscopic transcanicular DCR and balloon dilatation technique
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(2) Darmstadt

Purpose Combined surgery technique for high rate percentage of success in transcanicular dacryocystorhinostomy or a balloon dilatation technique alone.

Methods In this approach, transcanicular endoscopy with micro drill performing surgery like usual, dilatation of the opened channel with balloon dilatation and following injection of Healon 5 in the lacrimal duct are employed to reduced the risk of failure. This method is investigated through the two cases. Percentage of success of balloon catheter overall is estimated between 55% to 90% depends on the partial or complete obstruction. In the most of these cases of failure, cannulation was not possible because of complete obstruction. This problem might be solved by using micro drill before balloon dilatation technique.

Results In 2 cases, no more epiphora after 6 months follow-up were observed. (100% of success rate was achieved by 2 patients). However, more cases are needed to show the real success rate.

Conclusion Performing transcanicular DCR using micro drill and following balloon dilatation of the naso-lacrimal duct following an injectable product like Healon 5 or healon gel (by which will hold the nasolacrimal duct open and will stay in the duct for a short period) can diminish the failure of balloon dilatation technique. In this way the forming of synchia in all the duct from the punctum to the end of the distal duct will be reduced.

2444
Overview of the lacrimal stents
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Purpose Evaluation of the clinical usefulness CV balloon-expandable stent for maintenance of the opened (post-surgical) or dilated nasolacrimal duct.

Methods Under local anesthesia, injection of contrast for visualisation of the obstruction and fluoroscopic guidance placement of the CV balloon-expandable stent

Results Complications of the existing stents are as follows: concerning expandable metallic double stents(Song, 1993), where implanted under fluoroscopic guidance with severe epiphora after failed balloon dacryocystoplasty. During follow-up of 4-20 weeks complete blockage of the stent occurred in one of seven patients. Concerning Plastic stents (Song, 1994) 19 eyes - 79% complete resolution and 21% partial resolution of epiphora. Concerning Polyurethane stent, cause of failure was normal physiological reaction to foreign body; namely post-intervention infection, inflammation and fibrosis following obstruction (complete or partial), prolapse, occlude stents and stent malpositioning. Technical problems and complications during negotiation of the guidewire, the guidewire create a false passage, improperly positioned or partial outside the nasolacrimal system.

Conclusion Treatment of nasolacrimal stenosis with CV balloon-expandable stents can be a solution for a higher percentage of success and less complications due to the biocompatibility of the material and design. Also it obtains antibiofict material (will have much less inflammation, infection, and following obstruction) that release and prevent the fibrosis and indirectly the obstruction and blockage of the lacrimal duct. CV expandable metallic stents seem to be of value in the treatment of the complete or partial obstruction of the nasolacrimal duct.
**2445**

*Treatment of epiphora in infants*

*STIBN KRANJC B*
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**Purpose** To discuss the reasons of epiphora in infants and the natural history, treatment of lacrimal drainage system obstruction. In excluded excessive tear production epiphora means watery eyes in drainage failure. All parts of the lacrimal passages can be obstructed or anomalous, including lid malposition, skeletal, sinus malformation.

**Methods** Clinical practice with retrospective interventional case series in children (0-60 months) with epiphora. Descriptive analysis of a five year period, over 500 children. Complete resolution of symptoms counted as a patent tear outflow system.

**Results** Systemic associations were rare, a few children with Down syndrome had nasolacrimal duct obstruction. Only 7 of over 500 children who had undergone lacrimal duct probing needed dacryocystorhinostomy and intubation. All these children have presented recurrent dacryocystitis without orbital cellulitis. The microbial profile of the positive cultures consisted of Gram-positive and or Gram-negative bacteria, mostly of *Staph. aureus, Str. pneumoniae, Haemophilus influenzae*, all sensitive to chloramphenicol and ciprofloxacin and to at least 5 other out of 8-10 tested antibiotics. No complications of probing or surgery were seen.

**Conclusion** Probing and irrigation of lacrimal ducts is highly effective, safe and simple procedure even after the first year of age, though so far not determined whether probing really shortens the duration of epiphora. Spontaneous improvement is possible at any age. On the other hand early probing in dacrocystocele can reduce the incidence of dacryocystitis.

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**2446**

*Microsurgery of canalicular stenosis*

*SERRA F*
*Antibe*

**Purpose** To explain form the clinical finding which microsurgery of the canaliculi can be used to restore a spontaneous tear drainage in localized stenosis.

**Methods** Clinical and surgical cases demonstration. Methods: we will describe the different types of punctoplasty, the surgical repair of the stenosis located close to the puncta that can be reached by the direct approach and those close the lacrimal sac that require an approach via the common canaliculus.

**Results** The goal of this surgery is to recreate the continuity of the lacrimal mucosa in order to allow a spontaneous tear flow.
2451 Oxidative stress in the trabecular meshwork – Preventive effects of Prostaglandin analogues

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Purpose The trabecular meshwork (TM) of primary open angle glaucoma (POAG) is characterized by an increased accumulation of extracellular matrix, cellular senescence, and the loss of TM cells. One factor in the pathogenesis of POAG is oxidative stress. The goal of this study was to determine whether oxidative stress is able to trigger these POAG specific changes in cultured human TM cells and whether these changes could be reduced or prevented by the application of prostaglandin analogues (PA).

Methods Cultured human TM cells were stressed with hydrogen peroxide (H2O2) for 1 hour. Levels of fibronectin and VIM-P. 9 mRNA were analyzed by RT-PCR. Senescence-associated beta-galactosidase (SA-gal) activity was detected by histochemical staining. Cell loss was investigated by live dead assay. The effects of PA and benzalkonium chloride (BAC) on POAG typical TM changes were investigated by pre-incubation with solutions of bimatoprost, travoprost and latanoprost or its corresponding BAC concentrations of the either non stimulated or H2O2 treated cells.

Results H2O2 markedly influenced the mRNA expression of fibronectin (1.8 fold), VIM-P. 9 (0.4 fold) and increased SA-gal activity to 12 fold. Incubation with 600 μM H2O2 for induced 50% of dead cells. Pretreatment with BAC alone induced POAG typical TM changes in non-stimulated and H2O2 treated cells. These effects were reduced by preincubation with PA in H2O2-treated cells and to a lesser extent in non stimulated cells.

Conclusion Oxidative stress is able to induce several characteristic POAG TM changes in vitro and these oxidative-stress-induced TM changes can be minimized by the use of PA. Thus, prevention of oxidative stress exposure to the TM may help to reduce the progression of POAG.

2452 Aldose Reductase Inhibition Counteracts Diabetes-Induced Retinal Oxidative Injury, Gial Activation, and Apoptosis

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Purpose To access the role for increased aldose reductase (AR) activity in oxidative injury, glial activation, and apoptosis in retina of diabetic rats and high glucose-exposed cultured retinal pericytes and endothelial cells.

Methods Control (C) and STZ-diabetic (D) rats were treated with / without the AR-inhibitor fidarestat (F, 16 mg/kg /g, for 10 wks after 2 wks without treatment). The rate of apoptosis was assessed in flat-mounted retinas by TUNEL assay with immunoperoxidase staining, and nitrotyrosine (NT), poly(ADP-ribose) (PAR a marker of poly(ADP-ribose) polymerase activation) and glial fibrillary acidic protein (GFAP) expression in retinal sections by immunohistochemistry. Primary bovine retinal pericytes and endothelial cells were cultured in 5 mM or 30 mM glucose with / without F (10 microm) for 3-14 d. Apoptosis was assessed by TUNEL assay, NT and PAR by immunocytochemistry, and fluo and fid-2 expression by Western blot analyzes.

Results The number of TUNEL-positive nuclei (Mean ± SEM) was increased ~4-6 fold in D (207 ± 33 vs 49 ± 4 in C, p < 0.01), and this increase was partially corrected in D+F (106 ± 34, p < 0.05 vs D). The apoptotic cell number increased with prolongation of exposure of both pericytes and endothelial cells to high glucose. F counteracted high glucose-induced apoptosis, and NT and PAR accumulation in both cell types. Antipapoptotic effect of F in high glucose-exposed retinal pericytes was not associated with inhibition of fluo or increase in fid-2 expression (endothelial cell studies are in progress).

Conclusion AR inhibition with fidarestat counteracts diabetes-associated retinal oxidative injury, glial activation, and apoptosis.

Commercial interest

2453 Haptoglobin knockout mice exhibit morphologic changes consistent with iridocorneal dysgeneis and glaucoma

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Purpose To investigate the ocular phenotye of mice that lack the acute phase protein Haptoglobin (Hplk).

Methods Hplk mice were clinically examined at 16 weeks of age. Mice that showed macroscopic changes of the eyes were sacrificed and their eyes were enucleated, fixed in paraformaldehyde, histological sectioned and stained with hematoxylin and eosin. The diameter of the eye was measured and the histomorphology was evaluated and compared with eyes of normal wild type mice.

Results Clinical observation revealed that between 10 and 20 % of the Hplk mice exhibited gradual opacification of the cornea and shrinkage of the eyes. These mice were investigated. The eyes of Hplk mice were between 10 and 30 % smaller than eyes of normal mice. The eyes of Hplk mice showed rudimentary and more widely spread processi of the ciliary body. Moreover the anterior chamber showed goniocornea and irido-corneal adhesions, as well as attenuation of the trabecular meshwork. The neural retina showed a variable atrophy in ganglion cell layers in all Hplk mice and in the inner nuclear and plexiform layer in 80 %.

Conclusion Haptoglobin knockout mice have structural abnormalities of the anterior segment and neuretina comparable to human iridocorneal dysgeneis and developmental glaucoma.

2454 The localisation of erythropoietin and erythropoietin-receptor in the retinas of Goto-Kakizaki rats using immunohistochemical methods

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Purpose A number of data indicates that erythropoietin (EPO) exhibits neuroprotective and neurotrophic properties in the brain and in the retina. EPO has been recently described to be elevated in the vitreous fluid of patients with proliferative diabetic retinopathy or diabetic macular edema. The aim of our study was to describe the cellular localisation of EPO and its receptor (EPO-R) in the mammalian retina and to evaluate whether it is altered under diabetic conditions.

Methods Retinas of eight 10 weeks old type-2 diabetic Goto-Kakizaki (GK) and eight age-matched non diabetic control/Wistar rats were analyzed by immunohistochemistry in the study. Sections were cut from each specimen and stained with EPO and EPO-R specific antibodies. The labelling was visualized by the ABC method or by fluorescent antibodies.

Results Although the retinas of both groups showed similar and diffuse distribution of immunoreactions, the intensity of staining was variable among the specimens. The EPO-specific staining resulted a strong label in the ganglion cell layer and a less intense staining in the inner nuclear and pigment epithelium layers. The receptor-specific antibody labelled the same layers and structures, but the staining intensity was more robust. There was no significant difference regarding the staining pattern of either EPO or EPO-R between the diabetic and non diabetic retinas.

Conclusion Erythropoietin and erythropoietin-receptor are present in the retinas of GK rats although there is no significant difference in this early age as compared to non diabetic rat retinas. Further evaluation is necessary to assess the role of erythropoietin in diabetic retinopathy.
* 2455
Prevention of hypoxia/reoxygenation-, H2O2- and TGF-β2-mediated increase of alphaβ-crystallin and Hsp27 by the use of antioxidants

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Purpose Reactive astocytes in glaucomatos optic nerve changes are characterized by an increased expression of alphaβ-crystallin and heat shock protein 27 (Hsp27). Previously, we could show that hypoxia/reoxygenation (H/R), hydrogen peroxide (H2O2) and transforming growth factor beta 2 (TGF-β2) induced the expression of both Hsps. Our goal was to determine the ability of various antioxidants to prevent H/R, H2O2- and TGF-β2-mediated increase of alphaβ-crystallin and Hsp27 in cultured human astrocytes.

Methods Cultured astrocytes were incubated under hypoxic conditions (1% O2 for 4 hours) with subsequent reoxygenation (12 to 48 hours). Additionally, cells were treated with 400-800 μM H2O2 or with 10-100 μg/ml TGF-β2 for 12 to 48 hours. Expression of alphaβ-crystallin and Hsp27 was examined by real-time PCR and western blotting. To evaluate the effect of vitamin B1, B2, C, alpha lipic acid and anthocyanin, cells were preincubated with physiological concentrations before stress exposure.

Results H/R, oxidative stress- and TGF-β2 treatment markedly increased the expression of alphaβ-crystallin and Hsp27. This effect of H/R, oxidative stress and TGF-β2 was diminished when cells were preincubated with 100 μM alpha lipic acid and 100 μM anthocyanin. In contrast, preincubation of cells with vitamin B5, B12, C and E could not reduce the stress-induced increase of both Hsps.

Conclusion The antioxidants alpha lipic acid and anthocyanin are capable to reduce the H/R, oxidative stress- and TGF-β2-mediated increase of alphaβ-crystallin and Hsp27 in cultured human astrocytes. Therefore, the use of these antioxidants in glaucomatos patients may help to lower the incidence of characteristic changes in the optic nerve.

* 2457 / 203
Metabolic stress as a contributor to physiological opening of TRP channels in blowfly (Calliphora vicina)

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Purpose TRP channels in blowflies can be reversibly activated by metabolic stress. Activation can be caused by hypoxia or mitochondrial uncoupling and results in membrane depolarization. The changes that were observed in extracellular ion composition, photoreceptor membrane currents and changes in membrane potential. A specific signaling pathway opening TRP channels was rejected with findings that impairment of PIP2 under lack of ATP, results in activation of TRP channels. Even though the mechanism of metabolic stress TRP channel activation is elucidated, it remained unanswered what role of metabolic stress is needed to open the channels and whether under physiological conditions photoreceptor cells encounter the rate of metabolic stress that can significantly contribute to opening of TRP channels.

Methods Extracellular concentrations of [K+]o, [Na+]o and [Ca2+]o were measured using the ion-selective microelectrodes. The redox states of respiratory pigments (flavoproteins and cytochromes) in the eyes of blowflies (Calliphora vicina) were measured with time-resolved absorption spectroscopy and use of principal components analysis. Anaesthesia was achieved in 24 hour.

Results Changes in [K+]o, [Na+]o and [Ca2+]o appeared with latency of 8.06 ± 1.38 s. 10.38 ± 2.48 s and 13.11 ± 3.09 s, respectively (mean ± s.e.m., n=7). First detectable changes in redox state of cytochrome c respiratory pigment started at 1.79 ± 0.19 s and reached 39.4% ± 5.4% of the maximal reduction by the time changes in ionic composition were detectable.

Conclusion Our conclusion was that high degree of maximal redox state change is needed (39.4%) to open TRP channels, what is unlikely to happen under physiological conditions.

* 2458 / 204
Persistent pupillary membrane remnants attached to the anterior lens capsule

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Purpose To present an interesting case of bilateral persistent pupillary membrane remnants without visual acuity impairment. Persistent pupillary membrane remnants are an ocular "congenital anomaly" and often affect both eyes.

Methods A 12 year old dark-haired boy presented on a routine examination with dense and wide pupillary membrane remnants attached to the iris collarette and the anterior lens capsule bilaterally. In OD the remnants covered almost the whole pupillary area however leaving some clear islands and among them a central one. In OS the remnants did not cover the central pupillary area.

Results Uncorrected visual acuity was surprisingly good (9/10 OD and 10/10 OS). Although autorefraction was OD : +3.50 -2.25 X 180° and OS : +0.75 -0.25 X 170°; subjective refraction was OD : +1.25 X 90° providing 10/10 and Jager 1 and OS plano providing 10/10 and Jager 1.

Conclusion The characteristic of the pupillary membrane remnants is the attachment to the iris collarette and this is the most important differential diagnostic feature from pupillary membranes of other origin. Surgical removal of these membranes or YAG-laser membraneotomy is sometimes necessary in cases with severe visual impairment. The surprisingly good visual acuity in the present case is not suggestive of any attempt to remove these pupillary membrane remnants. Excellent near and distant visual acuity was preserved in the right eye due to a ‘pinhole effect’ of the central clear island which prevented the occurrence of amblyopia.
Physical principles and recent developments in in-vivo confocal microscopy

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Over the past two decades, the applications of in vivo confocal microscopy to the investigation of ocular surface anatomy and diseases in the living eye have been greatly extended. Confocal microscopy enables detailed investigation of tarsal and palpebral conjunctiva, central and peripheral cornea, tear film, and lids, as well as it allow evaluation of the ocular surface at the cellular level. High-quality imaging in both contact and non-contact modes has allowed new understanding of the functions of the ocular surface system, and in the coming years, such knowledge will become increasingly comprehensive and specific. Confocal microscopy may provide a link between well established ex vivo histology and in vivo study of ocular pathology, not only in clinical science but also in clinical practice. The purpose of this contribution is to summarize physical principles and recent developments using in vivo confocal microscopy of the ocular surface.

Distribution of dendritic cells its role in clinical differential diagnosis

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Langerhans cells (LC) are a critical factor in antigen presenting function in cornea and conjunctiva. These professional antigen presenting cells might be an important regulator of both innate and adaptive arms of the immune system. Corneal Langerhans cells are an issue of ongoing experimental studies. Until now was it impossible to investigate these cells in vivo. With the help of in vivo confocal microscopy was possible to show, that corneal LC are present both central and peripheral. Moreover, LC present as either large cells bearing long processes or smaller cells lacking cell dendrites, most supposed indicating mature and immature phenotype, respectively. The identification and distribution of the LCs was performed immunohistochemically. Quantitative evidence of LC in cornea and conjunctiva could be of clinical relevance in evaluation of wound healing, graft versus host disease, allergic and toxic drug side effects, etc.

Modern diagnostic possibilities of in vivo confocal microscopy and immunohistochemistry regarding LC distribution and density enable to compare the in vivo and ex vivo data and open a new horizon for clinical practice as well as experimental studies not only in human but also in veterinary medicine.

Implications of RLSM confocal laser scanning microscopy for the investigation of ocular anatomy

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Purpose Confocal imaging with the digital Rostock Laser scanning microscope (RLSM) based on the Heidelberg retina tomograph (HRT II) is a relatively new method for clinical in-vivo investigation of tissues in high resolution. We aim to analyse the use of this technique for morphological investigation from an anatomical point of view.

Methods The working principle, mode of image acquisition and resolution is compared to results that can be obtained by conventional histology and electron microscopy. Advantages and limitations of the RLSM technique are analysed and discussed.

Results The RLSM is based on a diode laser with a single wavelength and generates images from backscattered light of the tissue, which is influenced by refractive index, structure and depth within the tissue. Due to the maximal focal depth of about 1.5-2mm the investigation is best suited for ocular surface structures. It produces images of a resolution comparable to histology and overviews in scanning electron microscopy. The detection of fluorescent markers is presently not available in RLSM. As a distinct advantage of RLSM compared to conventional morphlogy, images can be obtained immediately in living tissue without the need of biopsy and tissue preparation, hence allowing real time functional analysis. Furthermore image stacks allow 3D image reconstruction for analysis of the spatial organisation of structures and time lapse allows the investigation of dynamic processes like blood flow and cell migration.

Conclusion RLSM is a valuable tool not only in clinical work but due to its high resolution and the ability of real time investigation, 3D reconstruction and time lapse images it is also very suitable for anatomical and cell biological research.

Alterations of conjunctival microanatomy in dry eye disease

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Purpose To demonstrate the possibility of imaging with in vivo confocal microscopy of pathological conditions in dry eye patients.

Methods Patient with dry eye and inflammation of conjunctiva were examined with Heidelberg Retina Tomograph II and Rostock Cornea Module. Superficial microanatomic structures were compared to those of healthy probands. Representative images of patients with dry eye are demonstrated and discussed.

Results In conjunctivitis and dry eye in vivo confocal microscopy discloses an irregular pattern of epithelial cells, as well as common signs of inflammation, such as infiltration with leukocytes and dendritic cells. It is also possible to see hyperreflective strands as sign of fibrosis. Pathological processes in meibomian glands can also be demonstrated by this method.

Conclusion Modern confocal microscopy allows imaging of microanatomic changes in dry eye disease and may help to verify the underlying pathology e.g. inflammatory processes.
**2465**

Anatomy of ocular lymphatic tissue in confocal laser scanning microscopy

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**Purpose**
A regular lymphatic tissue that belongs to the mucosal immune system of the body is present at the normal ocular surface. It is termed Eye-Associated Lymphoid Tissue (EALT). Due to its location in the mucosal ocular surface and its high cellularity it appears very suitable for confocal microscopy as attempted here.

**Methods**
Confocal investigation with the digital Rostock laser scanning microscope (RLSM) based on the Heidelberg retina topograph (HRT II) was performed in human and rabbit EALT and compared to histology and electron microscopy.

**Results**
EALT extends from the lacrimal gland throughout the conjunctiva into the lacrimal drainage system and consists of diffusely interspersed lymphoid cells and solitary lymphoid follicles. In the human conjunctiva it can be associated with the tubes or fawnor-like epithelial crypt inflodings. RLSM showed frequent lymphocytes as bright roundish spots together with bright cells of dendritic morphology in the epithelium and in the lamina propria. Lymphoid follicles in the rabbit conjunctiva contained numerous lymphocytes within a meshwork of connective tissue fibres. Towards the surface the fibre meshwork increased in density and appeared finally continuous with a bright layer conceivably representing the basement membrane. This contained numerous dark holes reflecting massive transmigration of lymphoid cells; somes times bright roundish lymphocytes occurred within the holes. Inside the epithelium M-cells containing groups of lymphocytes could be clearly detected by RLSM.

**Conclusion**
Eye-Associated Lymphoid Tissue (EALT) can be investigated by in-vivo confocal microscopy (RLSM). With this technique, structural and topographical details of EALT are seen in a resolution that also allows the detection of single antigen transporting M-cells.

**2466**

Laser scanning IVCM of the limbus

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**Purpose**
To present the recent development of in vivo confocal microscopes (IVCM) in the imaging with of the microscopic architecture of the the limbal structures in healthy and diseased human eyes.

**Methods**
Laser scanning IVCM analysis of human limbal tissues in normal, inflamed and limbal stem cell deficiency (LSCD)-affected eyes is investigated; images are compared with impression cytology.

**Results**
Ocular surface epithelia show a typical microscopic pattern of transition moving from conjunctiva towards limbus (with its unique structures such as Vog's piliades and crypts) and cornea, which needs to be considered when analyzing in vivo limbal epithelial features. IVCM provides images of the limbus and related epithelial transition zones which well compare with impression cytology of the limbal area. However LSC, located in protected basal crypts cannot be discerned by morphology. Confocal microscopy is a powerful morphological analysis tool, with limited real phenotypic cell and tissue characterization, but provides fine indications and suggests mechanistic understanding of what occurs in limbal disease affecting LSC function such as chemical burns, infections, chronic limbitis, ulcers and other diseases. The formation of corneal conjunctivalization is often preceded by identifiable microscopic signs of limbal damage such as inflammation, necrosis, loss of the transition pattern and epithelial shift. The limbus is also a specialized site containing resident dendritic cells and blood capillaries, and the confocal microscopic investigation of these structures is particularly helpful for the comprehension of the immune response of the ocular surface

**Conclusion**
The future applications of IVCM in limbal analysis will be based on a greater comprehension of the complex network influencing corneal and limbal function.

**2467**

The corneal module of the HRT-II: A new tool for assessing ocular inflammation

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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. In vivo confocal microscopy using the HRT II Rostock Cornea module’ (HRT II / RCM) provides better resolution and therefore outlines distinctively in vivo inflammatory changes occurring in the ocular surface. In vivo confocal microscopy is capable of providing corneal, conjunctival and limbal cellular details in different ocular surface diseases such as vernal keratoconjunctivitis, toxic keratitis, Thygeson’s keratitis or corneal neovascularization. In correlation with ex vivo impression cytology analysis, it thus constitutes an interesting aid in the diagnosis and management of numerous ocular surface conditions.
**3111**

Optical Coherence Tomography (OCT) and Ultrasonic Biomicroscopy in corneal diseases

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**Purpose** To present the utility of ultrasound biomicroscopy (UBM) and anterior segment optical coherence tomography Visante (AS-OCT) in the imaging of the human cornea.

**Methods** UBM and OCT analysis of healthy and pathological corneas are investigated, with the aim to define morphological/morphometric patterns of clinical relevance.

**Results** Cross-sectional scans provided by UBM and Visante OCT enable morphological investigations of the cornea, ocular surface, and anterior chamber. Particularly AS-OCT allows morphometric and quantitative analysis of the entire cornea, without invasiveness, together with corneal pachymetric maps. The anatomical relationships between the cornea and the adjacent anterior segment structures can be evaluated also in cases of loss of corneal transparency. Quantitative parameters can be obtained in cases of progressive corneal thinning, in degenerative and ulcerative diseases, descemetoceles, corneal oedema, and in the evaluation of post-surgical corneal anatomy, such as lamellar interfaces, keratoplasties, ocular surface reconstruction, and antiseptic membrane transplantation. Dynamic evaluations, over time, clearly allows clinicians to objectively assess variations of the corneal parameters (i.e. overall and sublayer thicknesses, lesion size, wound healing of stromal and transplanted tissues).

**Conclusion** UBM and AS-OCT are complementary analysis to biomicroscopy, and furnish morphometric and objective parameters also when the cornea is opaque, lying non-contact, and providing a higher resolution, AS-OCT should be preferred in the investigation of acutely diseased or surgically treated corneas. Cross-sectional morphology, tissue reflectivity assessment and precise measurements are the main advantages over traditional slit-lamp examination.

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**3113**

In vivo confocal microscopy of the cornea

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Confocal in vivo microscopy so far has not found its place in routine diagnostic of anterior segment disease. But progress has been made with recent developments mainly based on Laser scanning technology. This allows an optical sectioning of less than 5 μm thickness of high contrast and in well defined distances from the corneal surface as well as the examination of conjunctiva, sclera and the lid region. Information is given how to differentiate subpopulations of the epithelium (superficial, intermedial, basal cells) as well as overall epithelial thickness measurements. This is exemplified in normal individuals, contact lens wearers and patients with various kinds of ocular surface disease. Stromal keratocytes are displayed and quantified in different corneal layers. Information on keratocyte activation by higher densities of scatterers in the cytoplasm is possible, indicating increased metabolic activity. A more wide spread use of in vivo confocal microscopy will allow to judge on woundhealing in all kind of corneal disease and repair mechanisms such as corneal re-innovation following refractive corneal surgery. Cell populations such as Langerhans cells are displayed and quantified allowing information on immunological corneal activity in vivo. Woundhealing modulation in Glaucoma filtering surgery can be displayed on a cellular level and microcyst formation depending on the influence of Mitomycin C and 5 FU are demonstrated at various stages after surgery. This information may influence the postoperative management considerably. In vivo confocal microscopy recently has become easy to handle with improved imaging quality and possibilities of quantitative evaluation of ocular surface structures.

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**3112**

Corneal topography and aberrometry: contribution to the ocular surface to visual optics

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Aberrometry, topography and tomography for the anterior segment are a new application field for advanced diagnosis of visual loss and anterior segment diseases. The capability to investigate shape, anatomy and optical performance of eye is a key factor to identify the source for visual loss. This visual acuity (VA) impairment has to be related not only to loss of lines of VA but also to ales, glare, double vision and all symptoms that frequently the patients report and remain unanswered. Aberrometry allows to day not only to know if the optical quality is decreased from normality but also to identify the source of it: cornea, lens, all posterior segment. In addition MTF (modulation transfer function) is able to test in fraction of second the optical capability to transfer contrast of any image. These open new prospective studies, as is the only way to test contrast sensitivity in an objective way.

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**3114**

Impression Cytology in corneal and ocular surface pathology

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**Purpose** Impression cytology (IC) refers to the application of a cellulose acetate filter to the ocular surface to remove the superficial layers of the ocular surface epithelium. The SIS presentation describes the technique and uses of IC.

**Methods** These cells can be subjected to histological, immunohistochemical, or molecular analysis. Periodic acid Schiff (PAS), Papanicolaou or haematoxylin stains are commonly employed to delineate goblet cells and epithelial cell morphology. Cells harvested by IC can also be released as a cell suspension and subjected to FACS (fluorescein activated cell sorting) analysis. Proper technique is essential as the number of cells sampled can vary considerably.

**Results** Generally two to three layers of cells are removed in one application but deeper cells can be accessed by repeat application over the same site. Applications for IC include diagnosing a wide range of ocular surface disorders, documenting sequential changes in the conjunctival and corneal surface over time, staging conjunctival squamous metaplasia, and monitoring effects of treatment. It is also a useful investigational tool for analysing ocular surface disease with immunostaining and DNA analysis. It is non-invasive, relatively easy to perform, and yields reliable information about the area sampled with minimal discomfort to the patient. Complications such as removal of a full thickness sheet of corneal epithelium; subconjunctival haemorrhage due to rupture of vessels in patients with conjunctival laxity, or subconjunctival emphysema due to a tear in the conjunctiva have been encountered.

**Conclusion** Major ophthalmic centres should introduce this technique into routine clinical practice. A team approach should include the ophthalmologist, pathologist, microbiologist and immunologist.
# 3121

**Quantitative fluorescein angiograms**

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**Purpose** Clinical assessment of quantitative fluorescein angiograms based on novel instrumentation adaptation.

**Methods** Fluorescein angiograms were simultaneously performed on patient’s retinas and on standard cells composed of two sets of five standard fluorescein solutions mimicking the environmental conditions of the plasma and vitreous, respectively. Regular gray-scale fluorescein angiograms were converted into absolute concentration image maps, after a decoloration step for the separation of plasma and vitreous fluorescence contributions, taking into account the respective fluorescein quantum efficiencies. Diabetic retinopathy eyes, eyes before and 3 days after laser photocoagulation, and fellow eyes from patients with choroidal neovascularization were imaged using this new technique.

**Results** Repetitive measurements over patients clearly show the advantage of correcting for instrumentation setup. Comparison of scans taken before and after laser photocoagulation demonstrates background values within the same range and the expected increase over laser spots.

**Conclusion** Quantitative fluorescein angiograms compare favorably with conventional fluorescein angiograms by removing instrumentation setup bias and by allowing absolute concentration image maps to be built and comparable. Furthermore, the use of a non-linear color-coded map allows the detection of subtle differences not visible on regular fluorescein angiograms. It is realized that this first step needs to be complemented by other standardization procedures, e.g., by fundus reflectance.

# 3122

**Intermediate and combined forms of retinal telangiectasies**

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**Purpose** Idiopathic retinal telangiectasies are usually classified as Leber–Coats disease or juxtalavial telangiectasies. Intermediate and combined forms of these vascular disorders are rare and poorly described and understood.

**Methods** Clinical presentation of four cases of congenital telangiectasies associated to pigment epithelium disorders, two cases associated with optic disc anomalies, nine cases of multifocal progressive retinal telangiectasies and six cases of junctional congenital retinal telangiectasies.

**Results** Clinical presentations of four types of poorly described and understood congenital retinal telangiectasies considering fluorescein angiography pattern and therapeutic approach.

**Conclusion** Intermediate and combined forms of congenital retinal telangiectasies are highly interesting in order to attend a more accurate classification of these disorders based on presumed etiopathogenic mechanisms.

# 3123

**Prognostic tools for visual acuity in primary rhegmatogenous retinal detachment**

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**Purpose** To study the influence of central retinal artery (CRA) blood velocities, intraocular pressure (IOP) and Endothelin-1 (ET-1) on visual acuity (VA) of primary rhegmatogenous retinal detachment (RD).

**Methods** This is a prospective interventional clinical study of 66 patients undergoing scleral buckling (SB) procedure; with reattachment of the retina 8 months after surgery. CRA Doppler sonography parameters and IOP were measured before SB. Immunoreactive (IR) ET-1 was assayed (radioimmunoassay) in plasma and subretinal fluid (SRF). Snellen VA and fundus examination were checked before and 8 months after surgery. Stepwise multivariate linear regression analysis allowed us to find models for VA.

**Results** IOP and CRA end diastolic velocity (EDV) explains preoperative VA (R2=0.509, p<0.0001). Preoperative VA, existence of PVR, CRA EDV and plasma IR-ET-1 (in PVR) explains postoperative VA (R2=0.908, p<0.0001). But, preoperative VA, SRF IR-ET-1 and plasma IR-ET-1 improves last model in PVR.

**Conclusion** Preoperative VA, existence of PVR and CRA EDV explains 90% (88% in PVR) of the variability of postoperative VA, but a model with preoperative VA, SRF IR-ET-1 and plasma IR-ET-1 explains 90% of it in PVR/Supported by grants: FIS PI040460 and DGES 97/0528.

# 3124

**Microstructural changes of longitudinal retinal venous profiles measured by retinal vessel analyzer in systemic hypertension**

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**Purpose** The Retinal Vessel Analyser (RVA) observes vessels in their dynamic state non-invasively. We found earlier a significant increase in roughness along longitudinal vessel profiles with increasing age. Whether longitudinal retinal venous profiles in systemic hypertension (SH) are altered is investigated.

**Methods** 15 untreated SH-patients (age: 50±9; 11,9 years) and 15 age matched healthy volunteers were examined. After baseline assessment a monochromatic (530-600nm) rectangular flicker of 12.5 Hz was applied for 20 sec. In venous segments (1 mm length) vessel diameters were measured to obtain a longitudinal vessel profile.

**Results** Baseline average diameters and caliber range differed significantly in both groups (p<0.05) (median/interquartile, quartile): SH: 164.4/153.8, 168.4/MU: 33.5/21.6, 35.8/MU: control 146.4/32.3, 132.1/MU: 19.9/16.0, 2.56/MU. Average area under power spectra within the frequency band of 0.01-0.06 Hz differed significantly at dilation (SH: 0.171±0.11, 0.351); control: 0.366±0.28, 0.490); initial restoration (SH: 0.215±0.13, 0.359); control 0.382±0.303, 0.514) and restoration (SH: 0.252±0.12, 0.374); control 0.425±0.31, 0.498) (p<0.05).

**Conclusion** The veins in SH seem pre-dilated and p-stretched. The microstructure of longitudinal venous profiles in healthy volunteers and SH-patients does not change in different phases of the reaction. In longitudinal venous profiles in SH low frequencies are more expressed and mid frequencies are less pronounced than in the control group. These microstructural changes in vessel profiles in SH-patients might be an indication for alterations in the vascular endothelium, leading to impaired local perfusion and regulation.
**Free Papers: Retinal vascular disease**

**3125**

Diagnosis and follow-up of retinal detachment using a wide field scanning laser ophthalmoscopy.

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**Purpose** The wide field (2007) imaging system (Optos, Great Britain) using a scanning laser ophthalmoscope (SLO) enables to visualize a large part of the retina including the periphery. The present study was designed to evaluate the imaging system for diagnostic and training purposes in retinal detachment.

**Methods** All patients referred for retinal detachment from November 2006 to April 2007 were examined by one retinal surgeon who also performed the fundus drawing. An SLO image was taken by an orthoptist in training and interpreted by a resident, who were both 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 superiorty between 11 and 1 o'clock and inferiorly between 5 and 7 o'clock were missed on the SLO images. Two retinal detachments, one superior and one inferior, were missed on the SLO images.

**Conclusion** SLO image analysis enabled correct diagnosis of a retinal breaks and related retinal detachments in more than 7 out of 10 cases. The retinal detachment was detected on the SLO image in more than 9/10 cases. The wide field SLO imaging system is thus not suitable as a diagnostic tool of retinal detachment replacing the fundus examination, however it represents a potential tool for patient education and training of vision professionals.

**3126 / 330**

A new heavy internal tamponade in vitreoretinal surgery: an in vitro study

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**Purpose** To study the solubility of perfluorohexyloctane (Fl6H8) in silicone oil (PDMS 1000 – polydimethylsiloxane 1000) and to measure the viscosity and the specific gravity of this mixture.

**Methods** The solubility diagram of the mixture was obtained with the turbidimetric method, indicating the miscibility of Fl6H8 and silicone oil 1000 at all the useful temperatures. The viscosity was measured in steady shear conditions by using a controlled stress rheometer (Haake RS50) and a double cone/plate (DC 60/4) system, both at 25 and 37°C for different volume percent compositions of the mixture. The specific gravity was measured at 37°C using a digital densimeter.

**Results** A mixture of Fl6H8 v30% and PDMS 70 v% was found to be transparent and stable at all the useful temperatures. By combining these proportions of the two substances a resultant density of 1.06 g/cm3 was obtained. The viscosity of the 30% Fl6H8 mixture was 203 mPA.s at 25°C and 163 mPA.s at 37°C respectively.

**Conclusion** The ideal Fl6H8 and silicone oil mixture can be obtained combining 30% of Fl6H8 with 70% of silicone oil 1000.

**3127**

Profile of the Spanish ophthalmologist dedicated to retinal pathology. Preliminary results

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**Purpose** Define the profile of ophthalmologists dedicated to retinal pathologies.

**Methods** After approval of the Spanish Society of Retina and Vitreous (SERV) a data base of professionals dedicated to treat retinal diseases was created from three sources: members of SERV / V-R specialist according to pharmaceutical companies and suggested by well known V-R specialists. A confidential questionnaire was sent. We used Chi-squared and Fisher’s Exact test for qualitative variables and non-parametric Kruskall Wallis test for quantitative variables.

**Results** 490 possible V-R specialist were identified, obtaining 248 answers. The answers belonged to 154 teaching hospitals 61 %, non-teaching hospitals 22 %, teaching private institutes 5 %, non-teaching private institute 11 %. The mean population covered by public centres was 230,000 inhabitants, with a mean of 14.5 ophthalmologists and a median of 4 V-R specialists per centre. 82% of V-R specialist finished their training after 1980. 93.5% received V-R training: residency (81%), medical retina master (13.8%), surgical retina master (11.6%) and from expert colleagues (63.3%). The preferential clinical practice was: medical retina 23%, surgical retina 12%, both 60%, 93% attend V-R special consultation every week. 22% practice retinal surgeries, 14% cataract surgeries and 62% both. 79% practice vitreotomies (80/10 years), 84% intravitreal injections (2/week), 45% photodynamic therapy (PDT) (7/week) and 88% prescribe PDT (7/week). 90% is member of the Spanish Society of Ophthalmology.

**Conclusion** We approximately know the number of ophthalmologists dedicated to retinal pathologies, their specialist training and their clinical activity.
Epidemiology and clinical findings of exfoliation syndrome and glaucoma

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Purpose To review the most important information on epidemiology and clinical picture of exfoliation syndrome.

Methods The reported prevalence of exfoliation syndrome varies widely over the world, reflecting a combination of differences due to race, age, environment, ethnicity and other yet-to-be-defined reasons. Exfoliation syndrome is frequently associated with severe chronic secondary open-angle glaucoma.

Results The typical clinical identification of exfoliation can be relied upon the biomicroscopical detection of exfoliation material after pupillary dilatation. Whereas the classic picture of manifest exfoliation syndrome has often been described, the early stages of beginning exfoliation have not been yet defined. Next to the lens, exfoliation material is most prominent at the pupillary border. Pigment loss from the iris sphencter region and its deposition on anterior chamber structures is a hallmark of the exfoliation syndrome. Its ocular manifestations affect all of the structures of the anterior segment as well as conjunctiva and orbital structures. Exfoliation syndrome often is an asymmetric condition, though never truly unilateral. Consequently exfoliation glaucoma is usually asymmetric and it is typically associated with higher mean IOP levels, greater diurnal fluctuations in IOP and marked pressure spikes.

Conclusion Exfoliation syndrome is a condition of worldwide distribution and significance. It has characteristic clinical features and is currently the most important single identifiable risk factor for open-angle glaucoma.

Blood flow changes in exfoliation

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Purpose Evaluation of orbital blood flow parameters by using color Doppler imaging in patients with unilateral exfoliation syndrome (XFS).

Methods This prospective, comparative case-series included 33 unilateral XFS patients, and 33 age-sex matched control subjects. Color Doppler imaging was used to evaluate the XFS affected (Group 1), and unaffected (Group 2) eyes that were matched with the corresponding side (Groups 3, and 4) of control subjects. A masked radiologist obtained peak systolic velocity (PSV), and end diastolic velocity (EDV) of ophthalmic (OA), central retinal (CR), nasal posterior ciliary (NPCA) and temporal posterior ciliary arteries (TPCA) as well as mean velocity of central retinal vein (CRV). Resistive indices (RI) were calculated.

Results Orbital blood flow parameters had no statistically significant difference between Group 1, and 2 (p>0.05). OA of Group 1 had significantly low PSV and EDV, and high RI compared to Group 3. OA PSV, EDV, and RI for Groups 1, and 3 were 25.3±9.2 cm/sec (p=0.000), 5.9±2.5 cm/sec (p=0.000), 0.74±0.04 (p=0.000), and 35.1±11.9 cm/sec, 9.7±3.2 cm/sec, 0.69±0.07 respectively. The RI had a cut-off value of 0.72 between Group 1 (95% Confidence Interval [CI] for mean, 0.72 0.75) and Group 2 (95% CI:0.68-0.72). In Group 2, OA PSV and EDV also were lower than Group 3 while the RI value did not differ significantly. Blood flow values for CRA, NPCA, TPCA, and CRV did not show any significant difference between the groups (p>0.05).

Conclusion Ophthalmic artery hemodynamic parameters change in both eyes of unilateral XFS patients pointing out the systemic nature of the disease. The decrease in blood flow might be due to decreased vascular compliance through progressive buildup of exfoliative material in blood vessels.

New insights into pathophysiology of exfoliation

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Purpose Pseudoexfoliation (PEX) glaucoma is the most common identifiable cause of open-angle glaucoma worldwide comprising the majority of glaucoma in some countries. The underlying disorder, PEX syndrome, is a generalized process of the extracellular matrix characterized by the production and progressive accumulation of a fibrillar extracellular material in intra- and extracellular tissues. New insights into its molecular pathophysiology have increased the understanding of this disorder and the current concepts of pathogenetic mechanisms of PEX syndrome:glaucoma will be presented in this review.

Methods Basic science, clinical-pathologic correlations, and review of the literature.

Results Recent molecular biological data support the pathogenetic concept of PEX syndrome as a type of stress-induced elastosis, an elastic microfibrillopathy, with TGF-β1, increased oxidative stress, a dysbalance of proteolytic enzymes and their inhibitors, an impaired proteasome system, and low grade inflammatory processes being key factors in pathogenesis. Active involvement of trabecular meshwork cells in local production of PEX material in the juxtaglaucomalicular area may be the primary cause of outflow resistance and chronic pressure elevation in PEX patients. Additional pathogenetic factors include marked pigment dispersion, increased aqueous humor protein concentrations, and pressure-independent risk factors including an impaired ocular and retrobulbar perfusion.

Conclusion PEX syndrome may not only cause severe open-angle glaucoma but also a spectrum of other complications and imply an increased risk for cardiovascular disease. A thorough awareness of its pathologic features and its effects on ocular tissues is critical for an early recognition and accurate diagnosis.

Current concepts in the management of exfoliative glaucoma

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Purpose To review current concepts in the management of exfoliative glaucoma (XFG).

Methods Presentation of recently published studies on the success of medical, laser and surgical therapy in XFG.

Results The management of XFG should be different to that employed in POAG. Due to the worse 24-hour IOP characteristics in XFG, optimal control of 24-hour IOP is needed to improve long-term prognosis in XFG. There is cumulative evidence that new medications may show a different response in XFG compared with POAG. Directed therapy in XFG may employ fixed combinations or laser as initial therapy. Prompt surgical intervention is more often needed in XFG. The role of non-penetrating surgery in XFG requires further elucidation.

Conclusion Overall, the management of XFG remains a challenge.

Commercial interest
**3135**

Management of co-existing cataract and exfoliation

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**Purpose** To describe the way to manage coexisting cataract and pseudoexfoliative glaucoma (PEXG)

**Methods** Several videos will be shown, together with a step-wise approach to the possible problems these eyes usually have

**Results** We will show several useful surgical maneuvers to achieve success in these potentially complex cases

**Conclusion** When managed properly, the outcomes of surgery in eyes with coexisting cataract and PEXG can be improved
**3151**

Experimental models of ocular inflammation: an overview

**BODAGHIB B**

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Since the description of the role of different retinal antigens associated with intraocular inflammation in the early 70’s, a great number of experimental models have been developed in order to evaluate the pathophysiology of this autoimmune-inflammatory condition but also the efficacy of different therapeutic strategies. Experimental autoimmune uveitis is the most common murine model which has been used for these purposes. Disease development is mediated by Th1 CD4 cells specific for various retinal autoantigens. However, the role of these cells in the pathogenesis of uveitis remains poorly understood, because it is difficult to follow their activation and migration. Activated macrophages, attracted by cytokine secretion, also cause retinal damage due to the release of cytokines: oxygen and nitrogen metabolites, cytokines, and proteolytic enzymes. Even though different issues have been raised and major advances have been achieved, we are still lacking well-defined and appropriate relevance of these models for different human conditions. This is the main difficulty encountered by the research groups who try to use experimental models for the understanding and treatment of specific human entities. Only acute uveitis is obtained in the majority of classical models in rodents. Only a few models, in which a specific antigen may trigger the auto-inflammatory reaction without other associated factors, are available. It is highly important to revisit the experimental models of ocular inflammation in order to define the most appropriate ones for each human entity.

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**3153**

Vasoactive intestinal peptide loaded in liposomes: a new immunomodulatory agent for the local treatment of experimental autoimmune uveoretinitis (EAU)

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**Purpose** Local delivery of therapeutic molecules is a promising method to treat ocular diseases, while at the same time, reducing severe systemic side effects. We tested the efficacy of one intravitreal injection (IVI) of rhodamine labeled liposomes loaded with Vasoactive Intestinal Peptide (VIP-Rh-Lip) to reduce experimental autoimmune uveoretinitis (EAU).

**Methods** Injection of VIP-Rh-Lip was performed simultaneously with or 6 or 12 days after immunization with S-Ag in CFA. Therapeutic efficacy on EAU was determined by clinical slit lamp examination. Retinal tissue destruction was assessed by histological examination, inflammatory cells infiltration by immunohistochemistry. Intraocular cytokine production was determined by multiplex ELISA. The proliferation and cytokine secretion by cells isolated from inguinal LN were measured.

**Results** IVI injection of VIP-Rh-Lip simultaneously and 6 days after S-Ag immunization decreased EAU clinical score and protected retinal tissue from destruction compared to control animals. In treated rats intraocular inflammatory cytokines and NO production by intraocular macrophages were decreased suggesting they were deactivated. In vivo and in vitro T cell reactivity to S-Ag was reduced. IL-10 production by cells isolated from inguinal LN, re-stimulated in vitro in presence of S-Ag, was increased in VIP-Rh-Lip injected rats compared to control animals.

**Conclusion** A single intraocular injection of VIP-Rh-Lip is an effective therapeutic strategy to protect ocular tissues during uveitis, by reducing intraocular inflammation and by diminishing T cell reactivity at the systemic level.

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**3152**

Regulation of ocular inflammation—what experimental and human studies have taught us

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**ABSTRACT NOT PROVIDED**

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**3154**

Control of experimental uveoretinitis by specific or nonspecific regulatory T-cells

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**Purpose** We have previously described a murine model of experimental uveoretinitis. In this study, we have compared the therapeutic effects of polyclonal versus antigen specific CD4+CD25+ regulatory T cells after intravenous or intraocular injection according to the activation status.

**Methods** In mice expressing hemagglutinin (HA) in the retina after subretinal injection of (AAV), uveitis was induced by intravenous administration of 1 x 106 activated HA-specific T cells. These cells were obtained from purified Thy-1.1 TCR-HA CD25- cells and stimulated by irradiated BALB/c splenocytes and HA peptide for 4 days. At the same time or 4 days later, in vitro-activated or fresh HA-specific or polyclonal BALB/c CD4+CD25+ T cells were injected intravenously or intraocularly (IVI). A challenge was performed by intravenous activated HA-specific effector T cells, 21 days after the induction of uveitis. Intraocular inflammation was clinically and histologically studied in all animals.

**Results** Fresh CD4+CD25- T cells controlled uveitis only if they were specific for the target antigen (HA). Compared to intravenous injection, the effect observed after intraocular injection was obtained with low number of cells. Furthermore, protection against a challenge was achieved only after local administration of HA-specific regulatory T cells. Activated polyclonal BALB/c CD4+CD25+ T cells IVI controlled the disease but have no effect after a challenge.

**Conclusion** Regulation of experimental uveoretinitis may be obtained by using CD4+CD25- T cells. Specificity and activation status of these cells are critical points. In order to develop new in situ therapeutic strategies, in vitro-activated polyclonal Tregs are relevant cells.
Animal models of primary intraocular lymphoma

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Purpose Primary intraocular lymphoma (PIL) is a high grade non-Hodgkin lymphoma for which pathogenesis is still unclear. Few animal models exist in order to study this condition. We discuss and evaluate the contribution of different animal models available for intraocular lymphoma.

Methods Although intraocular lymphoma in humans is usually a B-cell lymphoma, most of existing models are based on T-cell proliferation. Recently, a major step forward has been realized with the development of new models of intraocular B-cell lymphoma. Moreover, to evaluate the role of the immune microenvironment in this condition, a murine model of intraocular B-cell lymphoma was developed in immunocompetent hosts after intravitreal injection of a syngeneic lymphomatous B-cell line.

Results These animal models of primary intraocular T-cell or B-cell lymphoma contributed to a better characterization of the molecular and cellular microenvironment of PIL. New therapeutic tools, such as the use of lymphokinase, or the manipulation of the immune microenvironment in order to control the tumor are being evaluated in these models.

Conclusion These more relevant experimental models will allow the development of new tools for the understanding of PIL pathogenesis and its therapeutic control.
**SIS: Non-melanocytic conjunctival lesions**

* 3161

**Non-melanocytic conjunctival epithelial lesions - overview**

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**Purpose**
To give an overview on the clinical presentation, differential diagnosis and treatment of non-melanocytic conjunctival epithelial lesions.

**Methods**
The current literature on non-melanocytic conjunctival epithelial lesions was reviewed.

**Results**
The non-melanocytic conjunctival premalignant and malignant epithelial lesions include actinic keratosis, dysplasia, intraepithelial neoplasia and squamous cell carcinoma. The traditional management was by surgical excision with clear margins and cryotherapy was added to ensure the complete destruction of tumor cells. During the last decade new approaches were suggested using topical chemotherapy with mitomycin C, 5-fluorouracil and interferon alpha-2b as well as photodynamic therapy.

**Conclusion**
New data is accumulating regarding the long and short-term follow up after traditional and newer treatment modalities for non-melanocytic conjunctival epithelial lesions. Based on this data, the common practice of the management of these lesions is changing from almost only surgery towards increased use of the different topical chemotherapy treatments.

* 3162

**Long-term results of combined treatment with excision and cryosurgery for malignant epithelial tumors of the conjunctiva**

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**Purpose**
To evaluate the long-term results of combined treatment with excision and cryosurgery for malignant epithelial tumours of the conjunctiva.

**Methods**
The medical records of patients diagnosed with conjunctival malignant epithelial tumours and treated with excision and cryosurgery were retrospectively reviewed. The demographic features of the patients, characteristics of the tumours, and the peculiarities of the recurrences were recorded. The recurrence-free survival was determined, and the logrank test was used to compare the recurrences according to histological classification.

**Results**
A total of 55 subjects (57 eyes), 19 female and 36 male, were included. Their ages ranged between 15 and 82 years (mean 55 +/- 14 years). Histological diagnosis was conjunctival intraepithelial neoplasia (CIN) in 26 (46%) and invasive squamous cell carcinoma (SCC) of the conjunctiva in 31 eyes (54%). All-eyes were treated with excision and cryosurgery with nitrous oxide probe. During the follow-up period of 6 months to 15 years, seven (12.3%) recurrences were observed. The success rate for patients with CIN and invasive SCC was 88.5 and 87.1%, respectively. There was no difference for recurrence rates between CIN and invasive SSC groups according to logrank test (P=0.68).

**Conclusion**
Over the long-term follow up, cryosurgery following excision is a successful method in the treatment of conjunctival intraepithelial tumours and invasive squamous cell carcinoma of conjunctiva with favourable outcome and minimal complications.

* 3163

**The square of squamous cell carcinoma of the conjunctiva to treatment with photodynamic therapy**

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**Purpose**
Photodynamic therapy (PDT) was originally developed for tumour treatment. Based on initial promising animal studies, we investigated the clinical use of PDT for treating conjunctival neoplasia.

**Methods**
Patients with newly diagnosed or recurrent conjunctival neoplasia / squamous cell carcinoma of the conjunctiva (SCC) were treated as part of an interventional case series. Patients received one to three treatments of 6 mg/m2 body surface area verteporfin (Visudyne; Novartis Ophthalmics, Switzerland) intravenously. The light dose was calculated as 50 J/cm2. All tumors were irradiated 1 minute after a bolus injection. Patients were followed weekly and then in monthly intervals up to 1 year. Retreatment was performed as early as one month after the initial treatment based on clinically and angiographic exam.

**Results**
Angiographic occlusion of the tumor vasculature was usually observed one week after treatment. Signs of tumor regression were seen in all patients one month after treatment. Highly vascularized (papillomatous) lesions showed the most dramatic response to treatment. PDT was well tolerated with only minimal side-effects including mild ocular irritation, subconjunctival hemorrhage, chemosis and one case of infusion related back pain. No conjunctival scarring, thinning or scleral thinning were observed following treatments.

**Conclusion**
Photodynamic therapy could potentially offer a less invasive approach to treating non-melanocytic conjunctival lesions in a subset of patients. However, larger studies are warranted to assess optimal treatment parameters and long-term tumor control.

* 3164

**Topical 5-fluorouracil treatment and topical interferon alpha-2b treatment for conjunctival malignant lesions**

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**Purpose**
To review the indications and limits of topical chemotherapy with 5-fluorouracil (5-FU) and interferon alpha-2b (INF) in the treatment of squamous cell neoplasia of the ocular surface.

**Methods**
The author reports the data of his long-term prospective studies where 5-FU or INF were applied as primary or adjuvant therapy in the treatment of squamous cell tumors of the conjunctiva. Clinical, cytological and more recently confocal microscopy data are presented and critically analyzed.

**Results**
5-FU represents the first choice topical chemotherapy for squamous cell tumors of the conjunctiva (including the entire spectrum of this disease) as sole treatment (in multifocal tumors, recurrent neoplasia or selected cases) or adjuvant treatment (following surgery and reconstruction with amniotic membrane). INF may be also used, when tumors are less aggressive.

**Conclusion**
Topical chemotherapy is becoming the standard in the intergrated therapeutic approach to conjunctival squamous cell tumors, limiting the indications for more aggressive therapies (radiotherapy).
3211

Densitometry of meibometer samples from normal and dry eye subjects

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Purpose: To compare the original and modified techniques of meibometry and scanning/densitometry of lipid blots, on normal and dry eye subjects.

Methods: A hand-held loop of plastic meibometry tape was pressed on the lower lid margin (15sec contact at 15g load), or fixed in the prisms-holder of the Goldmann tonometer arm (1sec contact at zero load) to obtain meibomian glands from right and left eyes of the same subject. The tape blots were scanned with the IRIS Pen hand-held scanner to obtain digital images of the blot area and background (unblotted) tape. Mean pixel density of the blot area was found with the NIH image software and mean background density subtracted. Some blots were measured with both the original Meibometer instrument and by scanning.

Results: Comparison of blot densities by the Meibometer and scanning/densitometry on 25 normals showed no significant difference, irrespective of background levels, making the Meibometer unnecessary. A good correlation was also seen between the original and the modified application techniques despite the lower pick-up of the latter. This is also preferred where patients cannot tolerate the 10sec application without blinking, as in many dry-eye cases. Mean pixel density was lower in 14 meibomian gland dysfunction patients (6 with, 8 without aqueous tear deficiency) than in normals, although the difference was not significant; readings were not always reproducible due to scanning errors, possibly indicating local tape saturation or composition-dependent effects.

Conclusion: IRIS Pen densitometry gives comparable results to the original Meibometer; so the Pen and a laptop computer can be used in the clinic or in field studies. More work is needed on scanning reproducibility and the effect of other environmental factors.

3212

Correlation between diagnostic tests guides the development and use of a dry eye diagnostic algorithm

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Purpose: The existence of large variations in results of diagnostic tests for cases of mild to moderate dry eye is widely recognised. The purpose of this study was to assess if there was correlation between existing dry eye diagnostic tests and attempt to develop a diagnostic algorithm.

Methods: A total of 91 subjects were recruited to the study. The tear film and ocular surface were evaluated by measuring tear volume using the phenol red thread test, tear breakup time using fluorescein, biomicroscopic examination of meibomian glands and impression cytological (IC) assessment of conjunctival goblet cells. Dry eye symptoms were assessed using McMonnies dry eye questionnaire and statistical correlations were assessed between all dry eye tests used.

Results: In this study cohort there were no severe aqueous deficient dry eye patients. Meibomian gland pathology, questionnaire (score of ≥14), IC and an alternative tear break up time (reference ≥7 seconds) demonstrated correlation, while no other tests demonstrated evidence of correlation. A diagnostic algorithm was devised using these tests alone, with maximum weighting placed on questionnaire with decreasing weighting assigned to IC, goblet cell grade, meibomian gland pathology and TIBUT.

Conclusion: We propose this development of a flexible probabilistic algorithm as a rational approach when diagnosing mild/moderate dry eye in large study groups. Until further studies have demonstrated the true sensitiveness and specificities of dry eye tests within the context of test sequences and particular patient cohorts are known, comparison of results between dry eye studies will remain questionable.

3213

Influence of platelets on the healing of dry eye of patients treated with topical eye drops of autologous Platelet-Rich Plasma (PRP)

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Purpose: To study the influence of high levels of platelets on patients suffering from dry eye and treated successfully with autologous PRP.

Methods: Platelet enrichment on autologous PRP of 16 patients (age 66±14) suffering from moderate to severe dry eye was evaluated and different dry eye parameters (symptoms, BCVA, fluorescein staining, impression cytology) were correlated with this value. 7 patients had lower that 1.3 fold platelet enrichment on their autologous PRP (low platelets (PLTs) group) and 9 had more than 1.75 fold platelet enrichment (high PLTs group).

Results: On the low PLTs group (7 patients), 50% of them showed a significant improvement of symptoms, 45% showed more than 1 line of BCVA improvement and 35% showed full disappearance of fluorescein staining. On the high PLTs group (9 patients), 78% of patient showed a significant improvement of symptoms, out of 10 patients evaluated for BCVA, 50% of them gained more than 2 lines of vision and 71% experienced a full disappearance of fluorescein staining.

Conclusion: Autologous PRP has been shown to be very effective on dry eye treatment. On this study we show that high levels of platelets present on this biologic product play an important role for the healing of dry eye. Other components of autologous PRP such as growth factors and vitamins should also help for improving these patients symptoms and main clinical signs.

3214

Nodular Degeneration

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Purpose: Recent studies suggest that Salzmann’s Nodular Degeneration can be treated effectively using excimer laser phototherapeutic keratectomy (PTK). However, superficial keratectomy (SK), a simple office procedure for removing the nodules, has shown similar post-operative results. The purpose of the study is to describe this procedure and present post-operative outcomes relative to improvement and/or stabilization of vision and relief of discomfort.

Methods: The study sample consisted of 36 eyes among 30 consecutive patients with symptomatic Nodular Degeneration. Twenty-five were female. Twenty-six eyes presented with decreased vision and discomfort, four with only decreased vision, and six with only irritation. All patients underwent removal of the nodules using the SK procedure.

Results: Thirty-three of the 36 eyes have follow up at the present time (average follow up, 1 year). Thirty-two of the 33 eyes have had improvement in vision, discomfort, or both. One has had improvement in vision but occasionally experiences mild discomfort. Two patients with initial improvement through 9 and 13 months had asymptomatic recurrences as of 27 and 17 months. The second patient has keratoconus and wore a hard contact lens, creating the recurrence which was removed before symptoms developed. At 22 months, there was no recurrence.

Conclusion: In this series, Nodular Degeneration has been successfully treated by SK without the addition of excimer laser therapy.
## Abstracts: Ocular surface and dry eye

### 3215

**Watch out if you tell it with flowers: Euphorbia latex keratitis**

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**Purpose** To report an unusual case of deep corneal ulceration due to the latex of *Euphorbia*.

**Methods** A 76 year old woman presented in emergency to the ophthalmology department with a red, painful eye and blurred vision 2 days after ocular exposition to the sap of *Euphorbia* species.

**Results** The best corrected visual acuity was 20/200. The examination in the slit lamp showed a deep central corneal ulceration, epithelial sloughing and Descemet’s folds. Cultures were sterile. The patient underwent local treatment, ciprofloxacin and pilocarpine dichlorhydrate 0,05% for 10 days with a corneal ulceration healing. A punctuate keratitis remained which disappeared under vitamin A ointment. Regular controls were performed. The best corrected visual acuity found 3 weeks later was 20/25. A review of the literature revealed that *Euphorbia* sap has an antineoplastic effect in rodents. This could be a cause of the delay in the corneal epithelial healing process.

**Conclusion** To our knowledge, this is the first case reported of a corneal ulceration after eye contact with the latex of *Euphorbia*. The clinical findings described in the literature varied from a mild keratoconjunctivitis to a severe keratitis with stromal edema, epithelial sloughing and anterior tears. We also encourage to wearing eye protection in those who work with this family of plants.

### 3216

**Menstrual cycle influences ocular surface parameters in normal and dry eye patients**

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**Purpose** We analyzed the changes of ocular surface parameters and symptoms in healthy and dry eye women over the menstrual cycle. To our knowledge, incomplete reports appear in the literature on this issue in healthy women still in the fertile period, and none on dry eye women of the same age. This to comparatively evaluate what then happens in peri- and post-menopause.

**Methods** 29 females in the fertile age and a regular 26-29 days menstrual cycle were included in the study. 14 subjects with and 15 without dry eye symptoms. Symptoms were scored by the validated questionnaire OSDI. Degree of dryness was evaluated with the Schirmer test I, Jones test, BUT, Ferning test, Tear Function Index (TFI) and conjunctival imprint cytology. Degree of inflammation was evaluated with conjunctival brush cytology and dosage of exudated serum albumin in tears. Hormonal cytology procedures were applied to exfoliated cells in tears. Patients were analysed during menstruation, in the follicular and in the preluteal phases over two consecutive cycles and results were statistically evaluated.

**Results** TFI, tear stability, surface dryness and inflammation were significantly related to the hormonal fluctuations in menstrual cycle, in particular to the estrogen peak occurring during the follicular phase, especially in dry eye patients. Subjective symptoms appeared to increase in the lutal phase, suggesting the presence of a premenstrual syndrome.

**Conclusion** The ocular surface is confirmed to be dependent from hormonal variations; clinicians would take into account these cyclic variations during the examinations of subjects still in the fertile age, with dryness symptoms.
# SIS: Inherited retinal disease: novel developments and treatment strategies

## 3221
**Retinal dystrophies & dysfunctions**

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**Purpose** To provide an overview of the current status of knowledge in inherited retinal dystrophies and dysfunctions.

**Methods** A case presentation format will be used to illustrate an overview of current insights into genotypes and phenotypes of generalised dystrophies and dysfunctions of the retina.

**Results** Much progress has been made in unravelling the molecular mysteries underlying generalised retinal dystrophies and dysfunctions, with a wide variety of functions attributed to proteins encoded by causative genes.

**Conclusion** Rapid progress is being made in the field of generalised genetic retinal disease, with the first gene therapy trial for Leber congenital amaurosis currently underway.

## 3222
**Macular dystrophies**

**MOORE T**  
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**ABSTRACT NOT PROVIDED**

## 3223
**Treatment strategies**

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**ABSTRACT NOT PROVIDED**

## 3224
**Electrophysiology as a diagnostic tool**

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**Purpose** To describe the use of electrophysiological investigation in the assessment of patients with inherited retinal disease.

**Methods** The techniques used to elicit ISCEV Standard ERGs, PERGs, EOG and mfERG will be discussed. In addition, reference will also be made to additional techniques such as ON-/OFF- response recording and S-cone ERGs.

**Results** Selected cases will be used to illustrate the value of electrophysiological investigation in the assessment and characterisation of inherited retinal disease. Particular emphasis will be placed on those disorders where the diagnosis is made by electrophysiology.

**Conclusion** The objective assessment of retinal, macular and RPE function using electrophysiological techniques is indispensable to the running of an inherited retinal disease clinic. Electrophysiology is likely to be the 'gold-standard' outcome measure in studies of potential therapeutic interventions.
**SIS: Intraocular pressure fluctuations in glaucoma: myth or reality**

* Circadian rhythm of intraocular pressure in healthy subjects and glaucoma patients
  
  **ROMANET JP, PALOMBI K, NOEL C, CHIQUET C**  
  Department of Ophthalmology - University Hospital, Grenoble

**Purpose** Intraocular pressure (IOP) varies and depends on many factors. Intraocular pressure variations throughout the nycthemeron (the full 24-period of a night and a day) are the most interesting for studying.

**Methods** With present techniques, it is impossible to measure continuously without waking the subject up. Thus, IOP must be hourly measured during 24 hours with a portable tonometer which allows short measurements in any posture, without requiring the subjects or patients to rise during the night.

**Results** Intraocular pressure depends on a nycthemeral rhythm, being higher at night than during the day in healthy subjects, with a nocturnal peak value (acrophase). In the same normal individual, several 24-hour measurements are identical. Each individual has his own 24-hour pattern of IOP. In glaucoma patients, however, the 24-hour rhythm of IOP was reversed, with values being higher during the day (a midday peak in IOP) than during the night.

**Conclusion** The time course of the nycthemeral curve of intraocular pressure is considered to play a role in the prognosis of glaucoma and allows to classify the type of glaucoma (POAG, NTG). Lowering of IOP is still the only option that is available for treating patients with glaucoma. Variations encountered in the individual nycthemeral IOP pattern must be taken into consideration to manage the most efficient treatment.

**Commercial interest**

* Large intraocular pressure fluctuations: a risk factor for glaucoma progression
  
  **ASRANIS**  
  Duke University, Durham, NC

**Purpose** We are aware of the detrimental effects of peaks of intraocular pressure (IOP) above the normal range. However, we have observed that many of our patients continue to experience glaucomatous progression despite their mean IOP being below what the physician considers an adequate target IOP for that individual. We studied the risk associated with diurnal IOP variations in open angle glaucoma.

**Methods** The patients who were enrolled in the study used the Zeimer self-tonometer to measure IOP over a period of 5 days. The readings were taken upon waking, noon, mid-afternoon, dinner-time and bedtime. Visual fields over the 10-year duration of the study were reviewed for progression. We performed statistical analyses on these readings to study the relative risk of disease progression.

**Results** Baseline IOP had no predictive value (relative hazard 0.98). The IOP range over multiple days was a significant risk factor for progression even after adjusting for office IOP age, race and visual field damage at baseline (relative hazard ratio 5.76). 88% of patients in the upper 25 percentile of IOP fluctuation, and 57% of patients in the lower 25th percentile of IOP fluctuation, progressed within 8 years.

**Conclusion** In this presentation, corroborating studies, common IOP fluctuations and proposed mechanisms for IOP fluctuations will be presented. Ways to control IOP spikes will be discussed and pharmacotherapy options will be explored. Diurnal fluctuations in IOP present a greater risk of visual field loss to the patient with glaucoma compared to mean IOP. By monitoring IOP more closely and by prescribing medication that offers the best control of IOP with the least amount of fluctuation, ophthalmologists can offer their patients the best therapy for glaucoma.

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**SIS: The relevance of nocturnal dip of diastolic blood pressure and the perfusion pressure of the optic nerve to glaucomatous disease progression**

* The relevance of nocturnal dip of diastolic blood pressure and the perfusion pressure of the optic nerve to glaucomatous disease progression
  
  **COLLIGNON N, COLLIGNON J**  
  Ophthalmology University Hospital, Liège

**Purpose** Although age, genetics and intraocular pressure (IOP) help to define the risk of glaucoma progression, there also exists glaucoma in which the pathogenesis is mainly the result of vascular disorders. To investigate the combined IOP and arterial pressure fluctuations risk factors for the glaucoma disease progression.

**Methods** The 24-hour ambulatory blood pressure and round-the-clock curve of the intraocular pressure were recorded in seventy patients: 51 primary open angle glaucoma (POAG) and 19 normal tension glaucoma (NTG). The mean systolic (SBP), diastolic blood pressure (DBP) and diastolic ocular perfusion pressure (IOPP ; DOPP -DBP - IOP) were calculated along with the nocturnal dip of systolic pressure and diastolic blood pressure. Two years disease progression were assessed for all patients by means of retrospective analysis of visual fields defects on repeated perimetricities. We performed statistical analyses on the readings to study the relative risk of the disease progression.

**Results** Abnormal (absence or increased) nocturnal dip of the diastolic blood pressure, large IOP fluctuations, circadian DOPP fluctuations were significantly associated with disease progression in POAG and NTG patients whereas no significant correlation was found for the other risks factors envisaged (i.e. zymopha,benderly,vasoquant,...).

**Conclusion** Circadian DOPP fluctuations was the most significant clinical risk factor for the glaucoma disease progression. Our data are in line with several population based epidemiologic studies that will be presented during the talk. By monitoring this DOPP that does not require either sophisticated or invasive investigations, it would be wise to take this factor into account in the clinical evaluation of our glaucoma patients.

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**SIS: Diurnal IOP curve versus 24-hour IOP curve: when and how to do it in practice**

* Diurnal IOP curve versus 24-hour IOP curve: when and how to do it in practice
  
  **KONSTAS AGP**  
  1st University Department of Ophthalmology, Thessaloniki

**Purpose** To review the role and utility of 24-hour, or diurnal IOP monitoring in the management of glaucoma.

**Methods** Current glaucoma practice generally involves single IOP readings at each visit owing to time, convenience and cost considerations. A single IOP measurement however, gives data for only one minute of the day and does not reflect the dynamic equilibrium of IOP during the 24-hour cycle. There is significant unpredictability of single IOP measurements and poor correlation between single IOP readings and peak or fluctuation of 24-hour IOP. 24-hour or diurnal IOP monitoring can provide valuable information for the diagnosis and management of glaucoma.

**Results** Although 24-hour monitoring is especially helpful in advanced or progressive glaucoma a simplified diurnal curve (IOP readings at 10:00, 14:00 and 18:00) can be successfully employed in most glaucoma cases.

**Conclusion** A simplified diurnal curve can provide reasonable quality of IOP information and help us setting a better target pressure. Better understanding of the prognostic value of 24-hour IOP characteristics and the ideal target 24-hour IOP are needed in the future.
**Which glaucoma treatments best flatten the IOP fluctuations?**

**Conclusion** Intraocular pressure (IOP) is the major risk factor for glaucoma and its progression. Since the beginning of its medical treatment, reducing IOP has been the only way of treating the majority of glaucoma cases. The role of IOP fluctuations as an independent risk factor for glaucoma progression is controversial, even if data on the beneficial effect of a stable circadian IOP control have accumulated in the recent literature. The circadian evaluation of IOP is therefore an useful complement of the appropriate diagnosis and follow-up of glaucoma. After many years of medical treatment of glaucoma based almost exclusively on the effect of beta-blockers in reducing the production of aqueous humour, other molecules with different mechanisms of action and pharmacodynamics have been introduced in large number. In most cases, different classes of drugs are characterized by different duration of their effect, with variable timing of peaks and troughs and, as a consequence, different dosage. Moreover, some drugs may show reduced efficacy during the 24 hours, as it is the case for beta-blockers during the night. Taking this into consideration may help to control IOP fluctuations, thus improving the control of glaucoma and reducing progression. Combining different drugs with different mechanisms of action may add further advantages. The aim of the presentation will be to review recent studies aimed at evaluating the role of medical, laser and surgical treatments in the control of IOP fluctuations in glaucoma patients.

**Commercial interest**
**3241 Foundations of lens ion transport**

**DELAMERE NA**  
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**Purpose** Prof. George Duncan began work on the lens almost 40 years ago. His studies laid the foundation for our understanding of the mechanisms that regulate lens electrolyte content.

**Methods** In the late 1960s Prof. Duncan conducted microelectrode and tracer flux studies that illustrated a link between ion transport, osmotic balance and lens transparency.

**Results** His work defined the permeability properties of lens membranes and the requirement for equilibrium between passive ion motion and active ion transport. Later, Prof. Duncan and his colleagues at the University of East Anglia carried out an analysis of human cataractous lenses that revealed abnormal sodium, potassium, calcium and water levels in lenses with cortical opacification. Prof. Duncan presented the argument that the lens swells, calcium rises and cellular organization is disrupted if active sodium potassium transport fails to balance cation leakage. Over the course of several years, the University of East Anglia group discovered evidence for a high degree of cooperativity between fiber and epithelial cells. Prof. Duncans discoveries became the foundation for later studies by his group and others that showed certain lens cells are specialized for active ion transport and shift ions for the entire lens.

**Conclusion** In recent years more has been learned regarding expression and regulation of Na,K-ATPase in lens epithelium and fibers. Recent studies by Taniya, Okano and Delamere (Ann. J. Physiol. 2007 In Press) point to alterations of Na,K-ATPase activity subsequent to stimulation of purinergic receptors. It is interesting that one of the earliest reports on functional purinergic receptors in lens epithelium came from Prof. Duncan and the University of East Anglia group.

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**3242 Calcium, the Universe and Everything**

**SANDERSON J**  
School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich

Calcium plays a pivotal role in lens physiology and pathophysiology. I will review research from the Norwich Eye Lab, which has been fundamental in establishing calcium and its function in the lens. Human cortical cataract is associated with a loss of lens homeostasis, with calcium overload a hallmark of this disease. Free calcium has also been found to increase in non-cataractous human lenses as a result of aging, suggesting that it may be an early event in cataractogenesis. Lens organ culture experiments, using bovine and human lenses, have shown that calcium overload is indeed fundamental in loss of lens transparency. Raised intracellular calcium initiated proteolytic events both in the soluble (crystalline) fraction and the cytoskeletal fraction, supporting a role for the calcium-activated protease calpain in the opacification process. In order to understand mechanisms of calcium overload, it is also important to understand the mechanisms of normal calcium homeostasis in the lens. This has been extensively studied, mainly in lens epithelial cells, with a particular emphasis on the role of the endoplasmic reticulum calcium store. Investigation of calcium signaling has, in addition to this, shown the lens to have a remarkable and diverse pharmacology, with functional expression of a plethora of receptors which signal via calcium including muscarinic receptors, purinoceptors and EGF receptors. Recent work in the lab has also extended this interest in calcium in the lens to other ocular cells types, where calcium signaling could have a role to play in other eye diseases such as retinal detachment and glaucoma. Investigation of calcium in the lens has demonstrated a central role in the pathophysiology of cortical cataract. By understanding the molecular mechanisms underlying cataractogenesis we move closer to the development of pharmacological strategies for the treatment of this disease.

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**3243 Sigma-1 receptor: a pivotal role in lens cell survival**

**WANG L, DUNCAN G**  
University of East Anglia, Norwich

**Purpose** Sigma-1 receptor (Sig-1R) has no known homology with any other receptors. Natural ligands are yet to be identified. Sig-1R appears to play a critical role in controlling cell proliferation. The human lens grows throughout life and uncontrolled growth after cataract surgery is responsible for Posterior Capsule Opacification (PCO). We have therefore investigated a possible role for Sig-1R antagonists in controlling PCO.

**Methods** The human lens cell line HFL124 expresses the Sig-1R and was employed for this study. Cells were maintained in MEMEM or EMEM supplemented with 5% Serum (FCS) at 37°C in 5% CO2.Sig-1R siRNA and scrambled siRNA were purchased from Ambion. Cell growth was assessed by measuring total protein and cell death by quantifying LDH leakage. mRNA level was assayed by RT-PCR. Protein level was measured by western blotting.

**Results** A dose-dependent effect of Sig-1R antagonist (rimcazole or BD1047) on cell growth was observed. 72 hours of transfection with siRNAs directed against Sig-1R reduced mRNA and protein levels by over 70 and 60% respectively. Subsequent incubation for 96 hours in 5%FCS gave a partial recovery of mRNA, but no significant increase in protein expression. LDH leakage assays showed that significant cell death occurred and was accompanied by an increased level of Caspase-3. The increases of pERK and pAkt stimulated with 10nM thrombin were inhibited by silencing Sig-1R, but not in cells exposed to scrambled siRNA. The recycling level of EGF receptor was significantly reduced by Sig-1R antagonists compared with controls.

**Conclusion** This study establishes a central role for the Sig-1R in lens cell survival through modulating the activity of growth factor receptor mediated cell signalling and growth factor receptor trafficking.

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**3244 Calcium signalling can be bad for you: thapsigargin and PCO prevention**

**WORMSTONE IM, WANG L, DUNCAN G**  
University of East Anglia, Norwich

**Purpose** Posterior capsule opacification (PCO) remains a significant clinical problem after cataract surgery. The Perfect Capsule device (Mibrella, Ltd) permits the introduction and subsequent removal of potentially toxic agents into the closed capsular bag. The present study aimed to determine the influence of thapsigargin (Tg) exposure on lens cell viability.

**Methods** HFL124 cells were routinely cultured in Eagle’s minimum essential medium (EMEM). DNA and protein synthesis rates were quantified by measuring the incorporation of [H] thymidine and [35S]-methionine. Longer-term growth was assessed by quantifying the increase in area over time of a circular patch of seeded cells. Human capsular bags were prepared from donor eyes and sealed with the Perfect Capsule device. 100nM Tg was introduced for 2 minutes. The bags were then perfused with EMEM and an IOL inserted before the bags were dissected and pinned to the base of plastic culture dishes and maintained in EMEM for 28 days.

**Results** Continuous exposure of >100 nM Tg to HFL124 cells for 24 hours significantly inhibited both protein and DNA synthesis. A 2 minute exposure of Tg resulted in a complete loss of viable cells following 4 days of culture. A 2 minute application of 100nM Tg to the capsular bag using the perfect capsule system did not immediately eliminate the residual lens cell population, however, by 4 weeks there were no surviving cells in Tg treated bags while a confluent cover of the posterior capsule was observed in controls.

**Conclusion** Thapsigargin inhibits cell proliferation and protein synthesis resulting in decreased survival rates. Application of thapsigargin at the time of cataract surgery for a 2 minute period using the Perfect Capsule system provides a promising means of preventing PCO.

**Commercial interest**
Growth factor signalling and regulation of lens cell differentiation

MCAVOY JW (1, 2, 3), STIMP RW (1, 3), ANG S (1), CHEN Y (1, 2), LOKCUTT F (1, 2, 3)

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Studies on explanted lens epithelial cells have been central to the identification of growth factor families that play key roles in determining the differentiated states of lens cells. In vitro models have shown that quiescent epithelial cells can be induced to differentiate down the normal fibre differentiation pathway by FGF, or induced to undergo an epithelial mesenchymal transition (EMT) that is a key feature of subcapsular cataracts (including PCO) by TGFβ. Central to the generation of these divergent phenotypes is reorganisation of the lens cell cytoskeleton. Recent studies in our laboratory have identified key roles for the Wnt growth factor family in regulating the lens cell cytoskeleton. Transgenic mice that overexpress the Wnt signalling inhibitor, secreted frizzled-related protein 2 (Sfrp2), specifically in the lens, develop cataract and microphthalmia and exhibit a severely disrupted lens cellular architecture. The epithelial sheet is reduced and disordered. The lens fibre compartment is severely disrupted and it is notable that fibres do not elongate and undergo directed cell migration to form sutures. This is reflected in the absence of aligned microtubules and disrupted expression/activity of components of Wnt/planar cell polarity (PCP) signalling cascades including small GTPases (Cdc42, Rac1 and RhoA) and phospho-INK. These results have highlighted an important role for Wnt/PCP signalling in orchestrating the development of the highly ordered lens cellular architecture.

Alpha-Crystallins and their role in cataract and other diseases

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In spite of intensive work trying to elucidate the 3 dimensional structure of alpha-crystallin, we still do not have a high-resolution structure. This is because alpha-crystallin is polymersized and cannot be crystallized. We can infer its structure from other small heat-shock proteins belonging to the alpha-crystallins family that were crystallized. To study its function in the lens, we are at present using a genetic approach. Xiaohua Gong and his collaborators have recently identified 2 mouse catactious mutant lines. One such mutation is aA-R54C, and the second is aA-Y118D. The two mutations cause a recessive and a dominant cataract respectively. The first mutation (aA-R54C) which is in N-terminal region of alpha-crystallin seems to affect lens epithelial and fiber cell during development, the aA-Y118D mutation which is in the “α-crystallin domain” of the molecule affect the interaction of alpha-crystallin with the other major lens crystallins namely beta and gamma-crystallins. This approach and these kinds of animal models will help us understand the multiple roles of the alpha-crystallin in the lens and in the other tissues. The involvement of alpha-crystallin in age related macular degeneration and various types of cancer will also be discussed.
Application of structured illumination techniques to corneal microscopy

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Purpose The microscopic study of corneal structures has always been a challenge for ophthalmologists and researchers. The inherent transparency of the cornea and the strong out-of-focus background are the main obstacles for achieving high-contrast images. In the past few years, traditional approaches based on the confocal principle have benefited enormously from the advances in scanning and detection technologies, but at the expense of an increasing complexity.

Methods As an alternative approach to the confocal principle, the use of different patterns (structured) of illumination to retrieve the in-focus features in an image may be advantageous for in-vivo imaging. It has been shown that structured illumination (SI) microscopy allows optical sectioning with at least the same resolution as standard confocal microscopy. Image acquisition is potentially faster, as optical sections of the whole field of view are obtained after acquisition of two or three widefield images rather than scanning every point in the field of view. In spite of it, the overall performance of these techniques may be affected by noise features that are particular to SI imaging.

Results We report on the application structured illumination microscopy to the field of ocular microscopy. To the best of our knowledge, this is the first time that corneal microscopy using structured illumination has been demonstrated. Encapsulated porcine eyes have been used to study SI-specific noise behaviour. Its implications for ophthalmic SI microscopy will be discussed.

Three-dimensional high-speed optical coherence tomography for the investigation of the cornea and anterior eye-segments

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Purpose Optical coherence tomography (OCT) has a history of more than a decade for posterior eye investigations. This posterior OCT has been optimized for retinal investigation and is not suitable for anterior eye examinations, although interest for the application of OCT to the anterior eye exist. Recently, anterior segment OCT has been introduced and its application fields are rapidly extending. However, these anterior OCT systems are based on a time-domain OCT configuration, which has relatively slow measurement speed; hence, it is difficult to have a full three-dimensional (3-D) tomographic dataset.

Methods Our group recently demonstrated ultra high-speed cornel & anterior eye segment OCT (CAS-OCT). This OCT system is based on Fourier domain detection and provides 20,000 A-lines/sec. This fast measurement speed enables a full 3-D dataset of the anterior eye within 2 sec. This 3-D dataset provides several useful applications: e.g. visualization of the internal structure of the bleb of glaucoma surgery. An en-face projection or en-face OCT slices are available from the 3-D dataset, which enables en-face assessment of corneal diseases whose extension directly relates to the patient’s visual acuity.

Results Using this 3-D CAS-OCT, we have examined 46 eyes with several types of pathologies including granular, Avellino and lattice corneal dystrophy, post LASIK corneal changes, keratoconus, irides nodules, anterior synechiae, angle recession, ciliary body detachment, scleritis, and several types of glaucoma surgery, trabeculectomy, trabeculotomy, and laser iridotomy.

Conclusion In the presentation, we introduce our CAS-OCT and show clinical outcomes form the above-mentioned examinations.

Optical coherence tomography of the choroid at 1050nm

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Purpose Optical coherence tomography is a well established imaging modality for the retina at 800 nm, but is limited in its capability to visualize through scattering tissue, like through cataract or into the choroid.

Methods Utilizing the lower scattering at longer wavelengths (1050 nm) high speed (17 kHz), high axial resolution (~7 μm) optical coherence tomography (OCT) demonstrates its ability to exploit the three dimensional anatomic microstructure of the choroid. Volumetric images, obtained with spectrometer based frequency domain OCT system are presented and compared with their counterparts acquired at the commonly used 800 nm range.

Results Tomograms of normals and patients with pathologic morphologic changes of the retinal structure as well as with cataract depict improved signal strength and image contrast at the longer wavelength. The deeper penetration allows reconstruction of the choroidal vascular structure despite strong scattering of superficial tissues at the retinal pigment epithelium, the choriocapillaris also including pathologic retinal thickening. Visualisation of modifications in the choroidal vascular structure associated with wet age related macular degeneration or diabetic retinopathy indicate the diagnostic potential of this technique. Furthermore, being well outside the spectral sensitivity of the eye the wavelength band at 1050 nm also allows for depth resolved profiling of reflectivity changes to directly monitor light stimulus related retinal activity, without interference due to the sampling OCT beam.

Conclusion Imaging at 1050 nm enables not only to track morphological but also functional changes of the retina.

Spectral domain OCT at 850 nm and OFDI at 1050 nm for retinal imaging in glaucoma and AMD patients

DEN BOER J
Wellman Center of Photomedicine, Massachusetts General Hospital, Harvard Medical School, Boston

Purpose We have realized a 150 fold improvement of the sensitivity over conventional time domain OCT technology allowing video rate in vivo retinal imaging without compromising image quality.

Methods Two different but similar technologies (SD-OCT and ODFD) provide high speed imaging at a wavelength of 850 nm and 1050 nm, respectively. An overview of the latest system developments will be presented. Data processing techniques to extract and quantify features such as retinal nerve fiber layer thickness, drusen volume, CNV size and volume, and subretinal fluid volume will be presented for a number of glaucoma and AMD patients.

Results Ultra-high resolution video rate SD-OCT at 850 nm and ODFD at 1050 nm offers many advantages in retinal imaging, maintaining high signal to noise while operating at a safe ocular exposure level.

Conclusion The unprecedented speed eliminates motion artifacts and the need to re-align A-lines, enabling accurate 3-D mapping of the retinal topography, and provides a shift from two-dimensional imaging to three-dimensional screening of large retinal volumes.
Motion of the scattering sites in the ocular fundus during transpupillary thermotherapy

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Purpose Our aim was to detect motion of scattering sites during transpupillary thermotherapy (TTT) using diffusing wave-spectroscopy technique

Methods The experiment was performed on a rabbit fundus. Motion of scattering sites in the fundus was monitored during TTT. A contact lens was used for easy fundus observation and guidance of the treatment and probe laser radiation. The treated area diameter was ± 4 mm. The optics of the diffusing wave-spectroscopy system were integrated into a standard ophthalmic microscope to allow a real time recording of the intensity of the light scattered by the heated scattering centres. Immediately before, during (at 5 sec intervals) and after TTT, autocorrelation functions were acquired. To exclude possible visible alteration of the tissues at the end of the treatment, an ophthalmologist observed the eye fundus.

Results The experiment showed that in the retina and RPE, temperature-induced scatters motion increases monotonically during TTT according to an exponential trend with time constant of ± 20 seconds. The choroidal diffusion coefficient showed a clear sharp step down in response to temperature rise. This trend does not reflect the trend of temperature but different boundary conditions or optical properties of the scatterers. Data showed also a slight increase in choroidal blood velocity upon heating of the fundus.

Conclusion These experiments showed the ability of the system to detect motion of scattering sites in the ocular fundus layers. We observed the temperature induced dynamics of retina and choroid scattering centers. Speculatively, the choroidal diffusion coefficient trend could be related to a retraction of the choroidal vasculature in response to increasing fundus temperature.
**# 3261**

**Treatment of ocular surface squamous neoplasia by topical Mitomycin C**

**FRUCHT-PERY J., PEER J.**

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**Purpose**

Ocular surface squamous neoplasia, which include conjunctival epithelial dysplasia, carcinoma-in-situ and invasive squamous cell carcinoma and which may involve also the cornea, were traditionally treated by surgical excision with the possible addition of cryotherapy, local irradiation, or external beam irradiation. Since 1992 we have treated the intraepithelial types of OSSN by topical Mitomycin C. Other centers have followed our steps. We will describe our and others’ experience using this type of treatment.

**Methods**

Our protocol for treating intraepithelial OSSN consists of applying 0.02% (0.2 µg/ml) Mitomycin in drops four times daily for two weeks, with additional such courses if needed, including a two week intermission between the courses.

**Results**

About 95% of our patients with intraepithelial OSSN responded to this treatment. About half of them needed only one course of two weeks, about 35% needed two courses, and the rest needed 3 to 5 courses. In those patients with corneal involvement, marked improvement of the visual acuity was noticed. Main side effects were conjunctival hyperemia and corneal epitheliopathy. No permanent complications were noticed.

**Conclusion**

Topical Mitomycin C is an effective and safe method for treating intraepithelial OSSN with relatively mild and temporary side effects. It can replace the surgical approach, especially for lesions that spread over large areas of conjunctiva and cornea and necessitate extensive excision.

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**# 3262**

**Treating conjunctival primary acquired melanosis with atypia by topical Mitomycin C**

**PEER J., FRUCHT-PERY J.**

Ophthalmology, Hadassah-Hebrew University Medical Center, Jerusalem

**Purpose**

Conjunctival primary acquired melanosis can appear with or without atypia. The lesions with atypia can become transformed to melanoma, and some consider them as melanoma in situ. The PAM lesion shows waxing and waning, and its boundaries are difficult to detect. Therefore, one can never be sure that the lesion was totally excised by the traditional method of surgical excision and cryotherapy. Since 1994 we have been treating our patients with PAM with atypia proven by biopsy, with topical Mitomycin C. Other centers have used the same methods. We will describe our experience in using this method of treatment.

**Methods**

The protocol we have been using consists of 0.04% (0.4 µg/ml) Mitomycin C drops four times daily for two weeks. Currently we apply 3 such courses, with two weeks’ intermission between courses.

**Results**

The vast majority of our patients showed partial or complete disappearance of the pigmentation. One patient had recurrence of the PAM and in one patient the recurrence was in the form of conjunctival melanoma. The main side effects were temporary conjunctival hyperemia and corneal epitheliopathy. In two patients the epitheliopathy was severe and long-standing, possibly due to limbic stem cell deficiency following a higher concentration of the Mitomycin C drop.

**Conclusion**

Topical Mitomycin C chemotherapy can be used as a good alternative to surgical excision and cryotherapy in treating conjunctival PAM with atypia, with reasonable side effects. We recommend not to use this method in treating invasive conjunctival melanoma.

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**# 3263**

**Using local carboplatin in the treatment of retinoblastoma**

**DESJARDINS I.**

Ocular oncology service, Paris

**Purpose**

Carboplatin is one of the most active chemotherapy in the treatment of retinoblastoma. Like other chemotherapeutic agents, it has potential mutagenic effects. To avoid these mutagenic effects, local delivery of carboplatin was studied.

**Methods**

Preliminary animal studies: Concentrations of carboplatin in the vitreous in mice proved to be higher after subconjunctival injections than after intravenous injections and to have a dose dependent effect on retinoblastoma vitreous disease (Harbour 1996, Murray 1996 and 1997, Hayden 2000 and 2004 and Yan Ke Xue Bo 2004). Coulomb controlled iontophoresis was also studied in mice by Hayden.

**Results**

Clinical results: a phase I/II study was performed by D Abramson on 13 eyes and reported in 1999. Vitreous and retinal tumor response were observed but no subretinal disease responded. Two children were treated in Cieze institute. One child with recurrent disease after external beam radiation including vitreous seeding was treated with 3 subconjugantal injections of carboplatin and silicone plaque brachytherapy. He is free of disease 9 years later. Another child with recurrent vitreous disease after external beam radiation was treated with carboplatin iontophoresis. No response was observed. Despite good initial response late recurrences can occur. Atrophy of optic nerve and severe orbital fibrosis have been reported and because of these severe side effects the use of subconjunctival injections cannot be recommended.

**Conclusion**

Local delivery of carboplatin may be one of the treatment modalities in the future if fibrin sealant (Van Quill 2005, Gorodensky 2005) or iontophoresis delivery can achieve good concentrations of carboplatin in eye with less orbital toxicity. Further studies are needed.

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**# 3264**

**Using intravitreal methotrexate for treating vitreoretinal lymphoma**

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**Purpose**

Vitreoretinal involvement occurs in approximately one-quarter of patients with primary CNS lymphoma (PCNSL), and in some patients with systemic lymphoma who may later develop CNS lymphoma. The traditional method for treating vitreoretinal lymphoma, which is the common type of intracocular lymphoma, is by external beam radiation, or intravenous or intrathacal chemotherapy; however, recurrences are common. We describe our ten years of experience in treating vitreoretinal involvement of PCNSL by intravitreal injections of methotrexate (MTX).

**Methods**

Biopsy-proven vitreoretinal lymphoma has been treated by intravitreal injections of 400 µg/ml methotrexate. According to our protocol, injections are administered twice weekly for 4 weeks, once weekly for 8 weeks, and then once monthly for 9 months for a total of 25 injections.

**Results**

Forty-four eyes of 26 patients have been treated; 7 patients had monococular involvement and 19 binocular. Twenty-three patients had B-cell lymphoma and 3 patients had T-cell lymphoma. In all patients remission of the intracocular lymphoma was achieved after 6.1 ± 3.5 injections (range 2 – 16 injections). None of the patients had an intracocular recurrence. Among the side effects, the most common were conjunctival hyperemia and corneal epitheliopathy, which usually subsided when the interval between the injections increased.

**Conclusion**

In our experience, treatment by intravitreal injections of methotrexate provides complete remission of vitreoretinal lymphoma with, so far, no recurrences and with bearable side effects, and can be considered as alternative treatment to radiotherapy and systemic or intrathacal chemotherapy.
**3265**

The potential of iontophoresis in introducing methotrexate and carboplatin into the eye

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(2) School of Pharmacy, Hebrew University-Hadassah Faculty of Medicine, Jerusalem

**Purpose** Intraocular injections of methotrexate have been used recently in place of the systemic approach to treat intraocular lymphoma and, sometimes, intraocular inflammation. Subconjunctival/subtenon injections of carboplatin have been recently used lately in some centers to treat retinoblastoma. In the last few years we have investigated the potential of iontophoresis for introducing these two drugs into the eye.

**Methods** Cylindrical agar gels served as a model resembling the eye for examining the transport of carboplatin in-vitro. In-vivo iontophoretic studies of methotrexate and carboplatin were performed on eyes of healthy rabbits, and the levels of the drugs were measured in different parts of the eye. The iontophoretic device we have employed is a portable, easy-to-use (Tonopen-like) system, operated by battery. The drugs were loaded into hydrogels of different sizes and shapes, and varying electrical currents were tested.

**Results** Iontophoresis increased significantly the levels of methotrexate in the various eye parts. In contrast, levels of carboplatin achieved by iontophoresis were not significantly different from our control mock iontophoreses.

**Conclusion** A short, low-current, non-invasive iontophoresis treatment using methotrexate-loaded hydrogels has potential clinical value in treating ocular inflammatory diseases and intraocular lymphoma. In contrast, carboplatin has high passive diffusion properties from the hydrogel into the ocular tissue, and iontophoretic application has no obvious advantage over local injection or application.
THEA lunchtime CIS: New evaluation techniques in ocular surface diseases

3271
In vivo experimental evaluation of cornea using high frequency ultrasound device
PISELLA P
Boulogne

ABSTRACT NOT PROVIDED

3272
The new imaging techniques for assessing ocular surface diseases
KRUSE FE
Erlangen

ABSTRACT NOT PROVIDED

3273
A new experimental approach to early signs of eye irritation after acute and repeated exposures on HCE (in vitro reconstituted human corneal epithelium); an evaluation of effects of some multidose tear substitutes
MELONI M
Milano

ABSTRACT NOT PROVIDED

3274
Comparison of antiallergic drugs using in vivo confocal microscopy for assessing late response of induced ocular anaphylaxis in the guinea pig
FERAILLE L
La Gaude

ABSTRACT NOT PROVIDED
### 3311

**Long-term results of LASIK in the correction of high myopia**

**LINDBOHM NM, TUISKU LS, TERVO TM**

Helsinki University Eye Hospital, Helsinki

**Purpose** LASIK is widely used in the correction of moderate and high myopia, even though reports on long-term results are few. We carried out this study to evaluate long-term results, stability and patient satisfaction after LASIK for high myopia.

**Methods** The study included 77 eyes of 47 patients who underwent LASIK for myopia of over -10 D in our hospital in the years 1999 to 2003. Preoperative and early postoperative data, including uncorrected and best corrected visual acuity and refraction, were collected retrospectively from hospital files. An additional examination with satisfaction query was performed 2 to 5 years after the operation.

**Results** After a follow-up of 2 to 5 years, 40% of the patients were within ±1.0 D from the original intended refraction, 70% within ±2.0 D, and 91% within ±3.0 D. Only one eye lost 2 Snellen lines of visual acuity, while 3 eyes gained two lines and the rest had no significant change. Regression was mild but statistically significant in long term, with mean spherical equivalent being -1.2 D at one month, -1.8 D at 6 months, and -1.9 D at two to five years. Nine eyes had mild postoperative complications which all resolved without permanent consequences, and none developed keratectasia. All except one patient were very satisfied with the results, and would have chosen the operation again.

**Conclusion** In conclusion, with careful patient selection and safety precautions, LASIK is a safe and relatively predictable alternative in the treatment of myopia of -10 to -17 D.

### 3312

**Comparison of corneal aberrations after myopic LASEK and LASIK surgeries using two different excimer lasers**

**CANADAS-SUAZUEZ P(1), HERNANDEZ-VERDERO J(1, 2), ROMÁN-GUINDO J(1), SANCHEZ-PINA J(1)**

(1) Visum Madrid, Madrid
(2) EUO Universidad Complutense de Madrid, Madrid
(3) Hospital Príncipe de Asturias, Alcalá de Henares
(4) Universidad de Alcalá de Henares, Alcalá de Henares

**Purpose** To compare changes in high order corneal aberrations (HOCA) after myopic LASEK and LASIK surgeries using two different excimer lasers.

**Methods** This prospective study included 124 eyes of consecutive patients divided into 4 groups: group 1 LASEK with Technolas 21 24 eyes, group 2 LASIK with Technolas 26 eyes, group 3 LASIK with Zeiss 37 eyes, group 4 LASEK with Zeiss 37 eyes. Uncorrected visual acuity (UCVA), best corrected visual acuity (BCVA) and manifest refraction were measured, in groups, before and 3 months after surgery. The topography data were used to calculate HOCA with 6 mm pupil size, before and 3 months after surgery.

**Results** There were no significant differences, 3 months after surgery, in BCVA, UCVA and in manifest refraction between patients treated with LASEK and LASIK regardless of the laser used. There was a significant increase in spherical, astigmatic, coma and total RMS aberrations in Technolas groups 3 months after surgery. They were lower in LASIK than in LASEK (p<0.001).

**Conclusion** Our results suggest that both lasers produced an equal increase in HOCA. Therefore, it depends on the surgical technique used. HOCA after LASEK were higher than after LASIK 3 months after surgery.

### 3313

**Long-term stability of LASEK performed to correct myopia in thin corneas**

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(2) Universidad de Alcalá, Alcalá

**Purpose** To study the stability of the refraction 15 months after myopic laser-assisted subepithelial keratectomy (LASEK) performed in thin corneas.

**Methods** We performed a retrospective study of 92 consecutive eyes that had undergone LASEK to correct their myopia and that had a preoperative corneal thickness ≤600 µm. Intraoperative mitomycin C (MMC) was applied when the ablation depth was ≤50 µm. We compared the residual spherical refraction between the 3-month and 15-month examinations, in order to detect a possible myopic change that would suggest secondary corneal ectasia.

**Results** Preoperative corneal thickness was 483.9 ± 140 µm (range 435 to 500). Preoperative spherical refraction was -3.60+2.40D. Preoperative cylinder was -0.90+1.10D. The residual sphere was -0.18±0.50D (range -1.25 to +2.00D). 3 months postoperatively and -0.13±0.7 (range -1.75 to +2.00D) 15 months after surgery (P<0.05). Uncorrected visual acuity was 0.97±0.2 and 1.0±0.2 respectively (P<0.05). Topography showed no signs of ectasia in any case.

**Conclusion** Myopic LASEK performed on thin corneas seems to obtain long-term stable refractive results, with no sign of ectasia 3 and 15 months postoperatively.

### 3314

**Stromal response to orthokeratology: an X-ray diffraction study**

**OWENS H(1, 2), HAYES S(2), MEKK KM(2)**

(1) Optometry & Vision Science, Auckland
(2) Optometry & Vision Science, Cardiff

**Purpose** To examine the impact of overnight orthokeratology lens wear on stromal collagen fibril diameter and spacing using synchrotron X-ray diffraction.

**Methods** Seven New Zealand White rabbits (10 eyes) wore orthokeratology lenses (Capricornia, Brisbane, Australia) overnight during a 2-week period to determine the effect of corneal refractive therapy on stromal collagen fibrils. To verify the effectivity of treatment, corneal topography (Medmont, Brisbane, Australia), corneal thickness (Allegan Humphrey pachymetry) and refractive error (retinoscopy) were measured prior to and after 3-4 days during the treatment period. Following completion of treatment, rabbits were euthanized and corneas immediately dissected and snap frozen for subsequent X-ray diffraction analysis every 1mm over the central 10mm horizontal corneal band.

**Results** Orthokeratology treatment induced an average change in refraction of 2.0 ±/− 1.3DS, and a topography-measured treatment zone diameter of 4.2 ±/− 0.7mm. The results from X-ray diffraction revealed no significant difference in collagen fibril diameter between treated and untreated eyes (P>0.05). Interfrillar spacing (IFS) was positively correlated with tissue hydration but no significant association between the change in IFS and refraction was apparent.

**Conclusion** The magnitude and time course of refractive change induced by orthokeratology in the rabbit eye is similar to that reported in humans, producing significant rapid and characteristic changes in corneal topography. Such specific changes in topography seem limited to the epithelial layers, with no parallel topographic modification evident in the underlying stromal architecture.
Diclofenac effect on conjunctival goblet cells density after LASIK

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(2) Vision - Institute Oftalmológico, Alicante
(3) Pathology & Surgery, Miguel Hernandez University, Alicante

**Purpose**
Dry eye is a common condition after LASIK surgery, and in previous studies we have demonstrated a great decrease of the goblet cell population after LASIK. Taking into account that dry eye syndrome has an inflammatory origin we performed this study with Diclofenac 0.1% after LASIK surgery.

**Methods**
40 patients scheduled for LASIK surgery were included into two groups. In 20 of them the flap was made with a mechanical microkeratome, M2 (Moria), and in the other 20 patients the flaps were made by femtosecond laser (Intralase). Topical Diclofenac 0.1% was applied twice a day for two months following LASIK surgery only in the left eye of the patients. Artificial tears were used 4 times a day in both eyes. Goblet cell count in conjunctiva and inflammatory cells were studied by impression cytology pre-operatively, at one, two and three months after surgery.

**Results**
Analysis by impression cytology of the ocular surface showed a decrease in the inflammation after surgery in the eyes treated with Diclofenac 0.1%. The goblet cell count in both groups decreased after LASIK surgery, however there were no statistically significant differences between left and right eyes of patients at any time in the goblet cell count in any of them.

**Conclusion**
Diclofenac 0.1% decreased inflammation in the treated eyes, however no differences were found with this treatment in the conjunctival goblet cell count.

**3316 LASIK-dry eye: fact or fancy**

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(2) Nuffield Laboratory of Ophthalmology, Oxford

**Purpose**
To review the evidence for dry eye after refractive surgery.

**Methods**
A review of the literature.

**Results**
Irritation and ocular surface staining after LASIK is termed LASIK-dry eye, treated with artificial tears and/or punctal plugs. Symptomatic recovery takes about 6 months. It has been attributed to a loss of afferent drive to the lacrimal gland and blink mechanism. Waking lacrimal secretion is stimulated by reflex sensory inputs from the ocular surface and nose. The rich innervation of the cornea, compared to the conjunctiva, determines that corneal incisions cause significant loss of drive from the ocular surface, gradually restored by corneal re-innervation. While LASIK-dry eye is a true entity, LASIK patients also suffer from LASIK-induced neuroepitheliopathy or LINE, a neurotrophic state due to interruption of trophic sensory nerves. The conditions are not mutually exclusive. The hallmark of LINE is the presence corneal staining confined to the flap and sparing the hinge region. Tear Break Up Time (TBUT) is normal or reduced and the Schirmer test is normal. The hallmark of LASIK-dry eye is staining of the interpalpebral conjunctiva, with or without interpalpebral-corneal staining, accompanied by a reduced TBUT and Schirmer test and increased tear osmolarity. Pre-existing tear film instability is a predictor of post-LASIK dry eye and pre-existing dry eye is a predictor of a chronic, post-LASIK dry eye state, associated with a greater risk of refractive regression.

**Conclusion**
Post-operative dry eye following LASIK or other refractive procedures is predictable and demands that all patients are assessed preoperatively for the signs of tear instability and dry eye. Preoperative detection permits a more accurate prognosis and the initiation of prophylactic measures.
### 3321

**Placental growth factor (PIGF) inhibition reduces choroidal neovascularization in a mouse model for age-related macular degeneration**

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2. Molecular Biology, Leuven
3. Ophthalmology, Baltimore

**Purpose:** To evaluate whether and through which mechanism PIGF blockade can inhibit choroidal neovascularization (CNV) in a mouse model of age-related macular degeneration (ARMd).

**Methods:** CNV was induced in mice by placing 3 Argon laser burns on the choroid. In a first experiment we compared CNV formation between PIGF knockout and wild-type mice. Secondly, 144 wild-type mice were injected every 2 days with 5, 10, 20 or 40 mg/kg of either an anti-PIGF antibody, an anti-VEGF receptor 2 (VEGFR2) antibody or an irrelevant control antibody. The CNV lesions were evaluated on histological cross-sections. The amount of endothelial cells, pericytes and inflammatory cells in the lesions were morphometrically analyzed after immunostaining for CD31, smooth muscle alpha-actin and F4/80 respectively.

**Results:** CNV formation was significantly reduced in the PIGF knockout mice. The dose-response experiment showed that the 20 mg/kg dose of anti-PIGF significantly reduced the number of vessels in the lesions as compared to control antibody (p = 0.045), although less than a comparable dose of anti-VEGFR2 (p = 0.027). Moreover, smooth muscle cell coverage was significantly increased in the anti-PIGF treated (p = 0.04) and reduced in the anti VEGFR2 treated mice (p = 0.03) as compared to control mice. Finally, a significant reduction in number of inflammatory cells was observed in the lesions treated with anti-PIGF (p = 0.017) but not in the anti-VEGFR2 treated mice (p = 0.33).

**Conclusion:** Anti-PIGF treatment inhibits CNV formation in a mouse model for ARMD. Both anti-PIGF and anti-VEGFR2 impair capillary growth, but in contrary to VEGFR2 blockade, anti-PIGF also reduces inflammation and promotes vessel maturation.

### 3323

**The PERSPECTIVES Study: pegaptanib efficacy results in multicenter, open-label study (Phase 3b/4) of early CNV in neovascular AMD over time, including vision function and QoL endpoints**

**Authors:** Hejsek L, PERSPECTIVES STUDY GROUP

**Purpose:** To describe the PERSPECTIVES Study - a phase 3b/4 trial designed to assess the efficacy and safety of pegaptanib (Macugen) for vision and quality of life (QoL) preservation in early or established choroidal neovascularization (CNV) secondary to neovascular age-related macular degeneration (NV-AMD).

**Methods:** This is a 102-week, open-label, multicenter trial being conducted in 18 countries. Patient assignment to early or established CNV groups is determined from baseline ophthalmic assessments, including angiography and fundus photos. Study eyes receive pegaptanib 0.3 mg every 6 weeks for 48 weeks and then every 12 weeks through week 102. Visual function assessments include visual acuity (VA), both ETDRS and modified Bailey-Lovie, contrast sensitivity, and reading speed. Subjects complete QOL questionnaires at baseline, week 54 and week 102.

**Results:** Enrollment is projected at 370 subjects (185/stratum) in order to provide 95% confidence intervals for the primary efficacy endpoint, mean change in best-corrected distance VA from baseline to week 54 for both classes of CNV lesions. As of January 2007, over 200 subjects have enrolled. Secondary endpoints include QOL scores from subject questionnaires, mean changes in distance VA from baseline to week 102, and mean changes in near VA, reading speed, and contrast sensitivity.

**Conclusion:** Subgroup analysis of the VISION trials indicated that treatment of early NV-AMD lesions may improve VA outcomes. The PERSPECTIVES Study was designed to provide a more comprehensive framework for evaluating treatment with pegaptanib 0.3 mg over 2 years in early versus more advanced stage NV-AMD.

**Commercial interest**

### 3324

**Safety of Macugen (pegaptanib sodium) for neovascular age-related macular degeneration (NV-AMD). After 3 years of experience**

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1. UZ Leuven, Leuven
2. Clinical Trial Group

**Purpose:** To evaluate the safety of Macugen in the treatment of NV-AMD after 3 years experience in the VISION trial.

**Methods:** Two concurrent, multicenter, double-masked studies included subjects with all angiographic subtypes. Macugen (0.3 mg, or 3 mg) or sham injections were given every 6 weeks for 54 weeks. Those initially assigned to Macugen were re-randomized (1:1) to continue discontinue therapy for 48 weeks; sham-treated subjects continued, discontinued, or received Macugen. At 102 weeks, subjects receiving Macugen 0.3 mg continued; those receiving other treatments or who received no treatment in year 2 were re-randomized to 0.3 mg or 1 mg (1:1: Safety endpoints included AEs, deaths, and lab values. Retrospective analysis compared fundus photos for RPE and optic disc damage of subjects receiving pegaptanib and sham.

**Results:** Macugen was well tolerated in year 3. In the primary safety population (n = 161) who received study medication in year 3 and active treatment during yers 1 and 2, AEs were mainly ocular (114 [71%]) subjects) and mild, with no evidence of increased systemic safety signals. Among 422 subjects treated with Macugen in year 3, regardless of previous treatment, 2 cases of endophthalmitis (0.06%) injection, 1 case of rhegmatogenous retinal detachment (0.03%) injection, and 0 cases of traumatic cataract were reported, and no evidence of new systemic safety signals was noted. No additional damage was found to the RPE or to retinal neurons in subjects receiving 0.3 mg pegaptanib vs sham.

**Conclusion:** Continuous treatment with Macugen over three years in patients with NV-AMD does not confer an increased risk for ocular or systemic AEs.

**Commercial interest**
Intravitreal bevacizumab in the treatment of refractory uveitic macular edema-A one year follow up observational study

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FOSTER CS (1, 2).
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Purpose To evaluate the long term outcome of intravitreal bevacizumab in the treatment of refractory uveitic macular edema (UME).

Methods Retrospective, non-comparative, interventional case series. SETTING: Massachusetts Eye Research and Surgery Institute. PATIENT POPULATION: All uveitic patients with macular edema who were refractory to conventional therapy and who were treated with intravitreal bevacizumab were identified and assessed. MAIN OUTCOME MEASURES: Best corrected distance visual acuity (VA) and optical coherence tomography (OCT) central macular thickness measurements. Correlative statistical analysis includes the use of the student’s t-paired test, Kaplan-Meier, and linear regression analysis.

Results 29 eyes of 27 patients with diverse uveitic etiologies were analyzed and followed up for a period of one year. Thirteen patients received a single intravitreal bevacizumab injection. Six patients required a second intravitreal bevacizumab injection, while ten patients received combination therapy of intravitreal bevacizumab and triamcinolone acetonide. Baseline mean LogMAR VA was –0.59. At one year the mean LogMAR VA was –0.42± 0.36 (P=0.0045). Baseline mean OCT central macular thickness was 383.66 microns. At one year the mean thickness value was 294.32± 108.77 (P=0.0007).

Conclusion Intravitreal bevacizumab is a useful and therapeutically beneficial agent in the treatment of refractory UME. Some patients will require adjunctive intravitreal bevacizumab injections or the use of combination therapy with intravitreal triamcinolone acetonide.

Efficacy and safety of pegaptanib sodium for macular edema secondary to Central Retinal Vein Occlusion (CRVO)

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Purpose To assess the efficacy and safety of pegaptanib in the treatment of macular edema secondary to CRVO.

Methods This phase 2, multicenter, double-masked, sham-controlled study included subjects with nonischemic CRVO of ≥6 months duration, baseline center point retinal thickness ≥250 µm, and best-corrected visual acuity (VA) of 65 to 20 ETDRS letters inclusive. Subjects were randomized (1:1:1) to intravitreous pegaptanib (0.3, 1 mg) or sham injections every 6 weeks for 24 weeks with follow-up through 52 weeks. Additional therapy was allowed after week 30 for losses ≥15 letters. Endpoints included mean changes from baseline in VA and retinal thickness.

Results Baseline characteristics were well balanced across study arms (0.3, 1 mg, n=33; sham, n=32). Mean change in VA from baseline at week 30 was +7.1, +9.9, and +12 letters in the pegaptanib 0.3 mg, 1 mg, and sham groups, respectively (P=.05 for 1 mg vs sham). At week 52, mean VA change was +7.5, +6.3, and -2.4 letters, respectively (P=ns). From baseline to week 52, fewer treated with 0.3 mg pegaptanib than sham lost ≥15 letters (9% vs 31%; P=.05) and more gained ≥40% (P=.01), 5 (P=.02) and 10 or 15 letters (P=ns). From baseline to week 52, mean change in center point thickness in the 0.3 mg, 1 mg, and sham groups was -295 µm, -216 µm, and -183 µm, respectively.

Conclusion This is the first randomized trial to show any treatment benefit for macular edema due to CRVO compared with sham. The anatomical and functional benefits provided by pegaptanib are consistent with the clinical benefit already presented with diabetic macular edema, and the ocular neovascularization of diabetic retinopathy and age-related macular degeneration.

Commercial interest
# 3331
**What have we learned about neuroprotection in the lab?**

**WHEELER LA, HARE WA, DONG CJ**

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**Purpose** Work both in vitro and in vivo models has provided insights into how and why retinal ganglion cells (RGCs) might die in glaucoma. In vitro and in vivo models have identified mechanisms of stress or injury-induced death of RGCs. Techniques used were cell culture, retinal whole mount and slice preparations to more complex in vivo models. These approaches have identified activation of the alpha-2 pathway and blockade of the NMDA receptor has important targets in neuroprotection.

**Methods** In vitro studies used high speed confocal Ca2+ imaging at the IPL of living rat retinal slices. Changes of cytosolic free Ca2+ were monitored with the Ca2+ dye fluo-4. Simultaneous recordings of ERG and single-unit RGC activity were obtained from rabbit retina flat mounts. Experimental ocular hypertension was induced by laser in rats (WoldeMusse, IOVS, 2001) and primates (Hare, IOVS, 2002).

**Results** At the IPL, the Ca2+ signal was suppressed in a dose-dependent manner by brimonidine and other alpha-2 receptor agonists. The action of brimonidine was blocked by yohimbine, an alpha-2 receptor antagonist. Application of NMMA to rabbit retinal flat mounts induced an increase in action potential spiking that was blocked by memantine. In vivo brimonidine and memantine reduced hypertensive injury to the optic nerve and retina.

**Conclusion** These findings suggest that a function of the retinal alpha-2 system is the regulation of synaptic transmission at IPL and that brimonidine may protect RGCs by preventing abnormal elevation of cytosolic free Ca2+ either in RGCs or in their presynaptic cells, or in both. Memantine is able to block NMMA-induced increases in in RGC spiking activity at concentrations that had little or no effect on retinal light signaling.

**Commercial interest**

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# 3332
**How to watch ganglion cells in the DARC?**

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(2) Glaucoma Research Group, Western Eye Hospital, London

**Purpose** Assessment of neuroprotection in vivo is difficult. DARC (Detection of Apoptosing Retinal Cells) is a newly devised, non-invasive real-time imaging technique using confocal laser scanning ophthalmoscopy to visualize single retinal nerve cell apoptosis in vivo.

**Methods** DARC allows longitudinal study of disease processes, which has not previously been possible. The implications of this novel technique include its application to glaucoma as a powerful new clinical tool with which to diagnose and identify patients with early disease, before they lose vision. Furthermore it may serve as a surrogate biomarker, providing real-time information that could dramatically reduce the duration of glaucoma clinical studies, which currently have to use visual field status as a key endpoint and determinant of outcome.

**Results** We have been able for the first time to image changes occurring in neuronal cell apoptosis over hours, days and months, and show effects depending on the magnitude of the initial apoptotic inducer in several experimental models of neurodegeneration.

**Conclusion** More importantly, by using the eye as a ‘window’ onto the brain, DARC opens the door to directly observing effects of therapeutic strategies in neurodegenerative disorders, by allowing for the study of potential neuroprotective drugs using meaningful endpoints that are based on the direct assessment of neuronal death in vivo. We have already assessed several neuroprotective agents using DARC including neuropeptide approaches.

**Commercial interest**

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# 3333
**How to monitor visual field progression?**

**VISWANATHAN A**

_Institute of Ophthalmology, London_

**Purpose** Although the scientific merits of trend analysis (PROGRESSOR) are encouraging, its suitability for randomized, controlled trials (RCTs) has not been widely addressed. This study assesses the performance of this type of analysis in the setting of an RCT.

**Methods** A collaboration between Moorfields Eye Hospital and the University of Parma attempted to determine the validity of trend analysis in measuring differences between glaucoma treatment interventions. Glaucoma patients with deteriorating vision, as indicated by negatively sloping trend lines, were randomly assigned to receive laser trabeculoplasty or anti-glaucoma medication treatment—in this case, brimonidine—to determine whether trend lines flattened out (denoting stabilized vision) or continued to slope downward (showing exacerbated visual loss).

**Results** Before and after treatment, the laser trabeculoplasty group demonstrated no discernable pattern, with pre- and postintervention graphs displaying negative slopes. With brimonidine, however, the trend reversed from negative before intervention to positive afterward, signifying an improvement in visual field status.

**Conclusion** These results are as yet unpublished but indicate that rate-based measures are indeed suitable for visual field outcome measurements in RCTs. Yet, further work is needed to assemble the optimal combination of summary, cluster, and pointwise methods for different trial conditions.

**Commercial interest**

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# 3334
**Does neuroprotection work in our patients?**

**GANDOLFIS**

_Dept. of Ophthalmology, University of Parma, Parma_

**ABSTRACT NOT PROVIDED**
**3341**

*Making sense of immediately sequential bilateral phacoemulsification*

LIU C
Swan Eye Hospital, Brighton

*Abstract*

Immediately sequential bilateral cataract makes sense, both for patient convenience, rapid rehabilitation, and health economics. However, there is still a stigma attached to it. We open by reviewing the advantages and disadvantages of operating on both cataracts at the same setting. We introduce the concept of conditional probability. We list the strategies available to reduce the risk of bilateral complications, including the option of not proceeding with the second eye procedure should the first operation be complicated.

**3342**

*Biometry issues*

SMITH G

To outline the biometric variables that we consider to be exclusion criteria when making the IOL power calculations for patients having simultaneous bilateral cataract surgery. I will also attempt to go beyond standard optimisation of biometry to yield results with a very tight standard deviation. I will then examine whether or not the biometric result in the first eye of unilateral surgery can be used to predict the outcome of the second eye.

**3343**

*Disruption of binocular vision following cataract surgery*

WHITE IES
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*Abstract*

**Purpose** The aim of this study was to assess the incidence of disruption of binocular vision following cataract surgery and to re-evaluate the role of Orthoptists in pre-assessment clinics for cataract surgery.

**Methods** A retrospective study was carried out of all patients who attended the hospital for cataract surgery between January 2003 and December 2003. The incidence of ocular motility disorders and diplopia, which had been recorded in the case notes was noted pre-operatively, two weeks post-operatively and if it was still persisting more than six weeks post-operatively. All patients with persistent diplopia/symptoms related to disruption of binocular vision had been referred for Orthoptic assessment.

**Results** In the period from January to December 2003, 2147 patients had cataract surgery, 93% of whom had surgery under local anaesthesia. There was a total of 17 (0.8%) cases with persistent disruption of binocular vision post cataract surgery. The aetiology of the binocular problems was divided into three groups: 1) pre-existing deviation and induced deviation. Treatment was by means of prisms, refractive correction, occlusion, Botulinum toxin and surgery.

**Conclusion** The incidence of disruption of binocular vision in those patients undergoing cataract surgery is not high. However, the symptoms that they have can be very difficult for the Orthoptist to eliminate despite a range of treatment modalities. The Orthoptist therefore has an important role in pre-assessment cataract surgery clinics in highlighting those patients in which there are potential binocular complications.

**3344**

*Health economics issues*

LEIVO T
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*Abstract*

**Purpose** To evaluate the cost and cost-effectiveness of the simultaneous bilateral cataract surgery compared to sequential bilateral cataract surgery.

**Methods** Design: Helsinki Simultaneous Bilateral Cataract Surgery Study. Prospective randomized comparative trial. Participants: 520 patients scheduled for bilateral cataract surgery were selected based on strict inclusion criteria to be operated in one session or sequentially one month apart. In both groups every second patient was included in the economic cost analysis. Methods: The costs were evaluated from the societal perspective, taking into account health and non-health care costs in both groups. The cost of surgery, complications, outpatient visits, medication, travel, patient’s and caretaker’s time and possible home nurse visits were evaluated. The cost data was obtained from the participant questionnaires and phone interviews at 3 months, hospital cost data and public statistics. The effectiveness was evaluated based on VF-14 and 15-D scores. Main Outcome Measures: The cost of simultaneous vs. sequential bilateral surgery per treated patient. The cost-effectiveness of simultaneous bilateral surgery compared to sequential bilateral cataract surgery. Economic sensitivity analysis was carried on all main results.

**Results** The preliminary results show, that cost per treated patient was lower in the simultaneous bilateral cataract surgery group. The effectiveness was similar in both groups.

**Conclusion** Simultaneous bilateral cataract surgery is cost-effective procedure. If additionally the treatment processes are tailored to simultaneous bilateral surgery, more cost savings can be achieved. The proportion of bilateral cataract patients operated in one session has a substantial effect on the use of health care and non-health care resources in the society.
**3351**

**Acute effects of the sigma-2 receptor agonist sarafemone on lens epithelial cells**

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(2) Department of Ophthalmology, Gothenburg

**Purpose**

Experiments were carried out to study the effects of sarafem on markers for apoptosis, oxidative damage and mitochondrial function in primary cultures of human lens epithelial cells (HLECs).

**Methods**

HLECs were incubated with 25 μM sarafem for 1, 2, 3, 4 and 6 hours. Caspase-3 was assayed in cell extracts with DEVD-AMC. Mitochondrial polarization was assayed with JC-1. Peroxide production was studied with DCF-DA and superoxide levels with hydroethidine. Glutathione levels were assayed with monochlorobimane.

**Results**

Sarafem induced a significant increase of caspase-3 activity after 6h exposure to 25 μM sarafem. Nuclear morphology examined with Hoechst 33342 showed signs of apoptosis after the same time intervals. A significant increase in the production of peroxide and superoxide were found up to 4.8 hours after administration of sarafem.

**Conclusion**

Sarafem, a piperidine analogue, was developed for the treatment of psychiatric disorders and is considered to be relatively nonotoxic. This study, and others, indicate effects on cell growth, apoptosis and production of ROS. The sigma-2 receptor may be a regulator of HLEC growth and apoptosis.

**3352**

**Dose dependence of alpha-tocopherol protection against in vivo ultraviolet radiation and associated alterations in lens redox balance**

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**Purpose**

To investigate the dose dependence of alpha-tocopherol protection against in vivo ultraviolet radiation (UVR) induced light scattering and associated alterations in the redox balanced in the albino rat lens.

**Methods**

Five groups of twenty, six-week-old female albino Sprague Dawley rats were supplemented with 0.5, 25, 50, and 100 IU/day alpha-tocopherol orally depending on group for 4 weeks. Then, each rat was unilaterally exposed to vivo in 8 kJ/m2 UVR 300 nm for 15 min. At 1 week after exposure to UVR, the rats were sacrificed, the lenses were extracted and homogenized. Lens total reduced (GSH) and oxidized (GSGG) glutathione, glutathione reductase (GR) and oxidase (GOx) were determined colorimetrically by enzyme reactions.

**Results**

Alpha-tocopherol caused a dose-dependent protection against UVR induced light scattering with a rate constant (1/τ) of 4.8 IU/day. The exposure to UVR caused a depletion of total lens GSH at low alpha-tocopherol supplementation that recovered towards higher alpha-tocopherol supplementation. The UVR exposure did not cause any alteration of total lens GSGG at any level of alpha-tocopherol supplementation. The UVR exposure did not cause any alteration of total lens GR or GOx activity at any level of alpha-tocopherol supplementation.

**Conclusion**

Alpha-tocopherol dose dependently protects against UVR induced light scattering. The protection of UVR induced light scattering is associated with a reduction of GSH depletion due to UVR exposure without any effect on total lens GSGG or GR- or GOx activity.

**3353**

**The presence of an active ETB1 receptor is essential for ETB2 induced relaxation of the iris sphincter muscle**

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**Purpose**

There are two subtypes of ETB receptors: ETB1 and ETB2. Stimulation of the latter relaxes iris sphincter. We investigated the interaction of the two ETB receptor subtypes in the iris sphincter muscle.

**Methods**

Rabbit iris sphincter muscle (n=55) were dissected, mounted on a vertical organ attached to a force transducer and precontracted. The effects of ETB stimulation through (i) Endothelins 1 (ET-1; 10e-10–10e-7M), in the presence of an ETA receptor antagonist (BQ-123;10e-5M; n=7) ii) sarafemone sc6 (SRTX-c1;10e-10–10e-6M; n=6) ii) IRL-1620 (ETB1 agonist; 10e-10–10e-7M; n=7) were evaluated. The effect of ETB stimulation was also evaluated in the presence of i) an ETA/ETB receptor antagonist, (PD145065;10e-5M: n=4) ii) an ETB1 receptor antagonist, (RES-701;10e-6M: n=7) iii) an ETB2 receptor antagonist, (BQ-788;10e-5M: n=6) iv) a NOS inhibitor, (L-nitro-L-arginine;10e-6M, n=7) v) a COX inhibitor (indomethacin;10e-6M, n=10). Results

ETB stimulation by SRTX-c1 or by ET-1 in the presence of BQ-123 promoted a concentration-dependent relaxation of the rabbit sphincter muscles maximal at 10-7M averaging 10.8±2.03% and 9.3±1.7% respectively, IRL-1620 did not relax the iris sphincter muscle (0.9±1.55%). The former was inhibited by RES-701 (0.09±2.23%) and BQ-788 (-2.20±2.04%). The effect was attenuated by PD145065 (5.70±0.98%) and by L-NNA (4.46±2.80%) or Indomethacin (2.3±2.93%).

**Conclusion**

ETB1 receptor stimulation relaxes the iris sphincter muscle, an effect that is mediated by the ETB2 subtype, through NO and prostaglandins release. However the presence of an active ETB1 receptor is essential for the activation of ETB2 receptor by sarafemone.

**3354**

**TNF-alpha and Interferon-gamma upregulate AQP4 protein expression in ARPE-19, a human retinal pigmented epithelial cell line**

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**Purpose**

Under physiological conditions, the outer retina is dehydrated by water movements through pigment epithelial (RPE) cells. However, during intracellular inflammation, water accumulation into the retina leads to the formation of retinal oedema. Accordingly, the knowledge of the type of aquaporins (AQP4) expressed by RPE cells and its regulation by cytokines is of crucial importance. Therefore, we studied the expression of AQP4 on the retinal epithelial ARPE-19 cell line and its regulation by cytokines.

**Methods**

ARPE-19 cells were grown for 24h in the presence or absence of 100 ng/ml interferon gamma (IFN gamma) or 30 ng/ml tumor necrosis factor alpha (TNF alpha). AQP4 expression was evaluated by semi-quantitative Western blot analysis and by immunofluorescence.

**Results**

Protein immunoblot of membranes from ARPE-19 cells were probed with an affinity purified anti-AQP4 antibody and an anti-glucose transporter 1. The immunoblots revealed the presence of a + 30kDa band, corresponding to the expected molecular weight of AQP4 observed in kidney membranes used as positive control. Immunofluorescence revealed a strong AQP4 labeling. AQP4 protein expression after 24 h treatment of ARPE-19 cells with 100 ng/ml IFN gamma or 30 ng/ml TNF alpha were respectively of 158 ± 23 % (mean ± S.E.M. n = 3, p=0.05) and 171 ± 10 % (mean ± S.E.M. n = 3, p=0.05) compared to untreated cells.

**Conclusion**

ARPE-19 cells constitutively express AQP4. In addition, AQP4 expression was significantly increased in response to IFN gamma and TNF alpha.
**3355**

**Efflux proteins of outer blood-retinal barrier**

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**Purpose** Blood-retinal barrier (BRB) restricts movements of drugs from blood circulation to the retina. Efflux proteins of retinal pigment epithelium (RPE), which forms the outer part of BRB, may have a role in this barrier. The aim of this study was to measure efflux protein (multidrug resistance associated proteins (MRP1-6) and breast cancer related protein (BCRP)) expression in RPE and to test activity of MRP5 in RPE.

**Methods** Expression of efflux proteins was studied by quantitative PCR, western blotting and immunohistochemistry. The activity of MRP5 was assessed by CDCFDA/CDCF efflux test and permeability assay. Probenecid was used as an inhibitor.

**Materials** included cell lines: ARPE-19, D407, HRPepC and tissues: bovine RPE/choroid and human eye specimens.

**Results** Expression of MRP1,4 and 5 was found in RPE both in RNA and protein level. In addition MRP2,3 and BCRP were expressed in D407 cell line. CDCF efflux was inhibited by probenecid in ARPE-19 cells indicating functional activity of MRP5 in ARPE-19 cells. The apparent permeability of CDCFDA/CDCF in bovine RPE/choroid tissue was ten times higher from neural retina to choroid compared to opposite direction.

**Conclusion** Particularly, we have shown pronounced expression of MRP1, 4 and 5 in RPE. Our results suggest that MRP5 may be active efflux transporter in RPE. MRP5 may function in physiology and also have impact on drug delivery to the posterior eye segment. Our findings may be important in the development of specific drug targeting to RPE and retina and to understand the function of BRB more comprehensively.

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**3356**

**Erythropoetin (epo) and the survival of retinal cells in diabetic retinopathy**

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**Purpose** EPO is approved drug for anemia treatment. Its antiapoptotic effects in animal models of glaucoma and retinal degeneration, when administered systemic or intravitreal are known. It provides neuroprotection and avoids cell damage in diabetic rats. It is upregulated in the retina in proliferative diabetic retinopathy (PDR). We studied serum EPO expression in diabetics and in normal non diabetic controls.

**Methods** Methods. In 58 type 2 diabetics, 24 with and 34 without retinopathy, of both sexes aged 64.24±11.64 years, EPO serum expression was determined by ELISA and compared to 44 non diabetic controls age and sex matched. Statistical analyses was applied (X2, Student t test).

**Results** Results: Serum EPO expression was highest in diabetics with retinopathy (15.15±11.44 mIU/ml high in diabetics without retinopathy, 10.08±6.63 p=0.043), compared to the controls 9.47±6.57.

**Conclusion** Conclusions: EPO serum upregulation in diabetic retinopathy supports endogenous over production seen in the retina with PDR. High EPO expression could be an attempt of nature’s protective mechanism. Human recombinant EPO is known to protect retinal neurons in various animal models of stress: ischemia/reperfusion; ocular hypertension/glaucoma (rats, mice, rabbits). EPO administration to RCS rats appears to exert (in vivo) neuroprotective effect on the photoreceptors. High EPO may be necessary to protect the damage of retinal tissue in diabetes. Constitutional EPO seems to be insufficient to give effective protection in diabetic retinopathy. Thus EPO could be an important therapeutic target molecule for the survival of damaging retinal cells.
**3361**

**Imaging of iris melanotic lesions and corneal tumors with three different high speed optical coherence tomography instruments**

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**Purpose** Should it be possible to detect anterior segment melanocytic lesions with anterior segment OCTs. When detected was special pachymetry possible to calculate our brachytherapy diagram.

**Methods** Three different IAS instruments were used, however at different times. In total 46 patients with light coloured eyes were analysed. The results were compared with already known clinical parameters and with anterior eye segment ultrasound when available.

**Results** Sixteen eyes were analysed using one infrared IAS, nine eyes using another infrared IAS and 15 eyes with a blue light IAS. Iris tumours were detectable in 90% using the infrared methods. With the infrared instruments we found the iris tumour or lesion in a high percentage so they could be analysed. With one of these infrared instruments we could compare the pictures with the ultrasound findings, but the pachymetry was even better than the ultrasound. In the selected group only anterior cilio-irido melanomas were detected. Corneal and conjunctiva tumorous lesions could be also in some cases detected.

**Conclusion** Conclusion: In this pilot study we demonstrated melanocytic lesions in light coloured eyes using the infrared anterior segment OCTs in a high percentage of the cases. Only anterior cilio-irido tumours were minimal positive. Using these instruments a new chamber angle pathology could also be found. Optimal special pachymetry for our brachytherapy calculation diagram was also good possible.

**3362**

**Conjunctival melanoma & histone deacetylase inhibitors**

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**Purpose** Conjunctival melanoma is a rare disease with a poor prognosis. Malignant conjunctival melanoma or primary acquired melanosis (PAM) with atypia are usually treated by surgical excision and cryotherapy. Topical mitomycin C (MMC) is also increasingly used for treating PAM with atypia. Histone deacetylase (HDAC) inhibitors can modify tumour growth and induce tumour cell death, and may be useful adjuvants for treating conjunctival melanoma. We examined the effects of HDAC inhibitors (sodium butyrate - SB, Trichostatin A - TSA) and MMC on the growth and survival of human conjunctival melanoma cells.

**Methods** Two conjunctival melanoma cell lines (CRMM-1 & CRMM-2), and primary human melanocytes and fibroblasts, were treated with SB (0.8mM), TSA (0.1mM), and MMC (0.20mg/ml) for up to 72 hrs. Viability was assessed using MTT assays and cell counts. Inverted and fluorescence microscopy was used to assess morphology and cell death (DNA stains).

**Results** HDACs and MMC significantly decreased cell growth and viability in a concentration and time dependent manner for both conjunctival melanoma cell lines. Low concentration HDACs (<2mM SB; <0.1uM TSA) reduced melanoma cell growth, associated with morphological differentiation, particularly in CRMM-1 cells. Melanocytes and fibroblasts remained viable with HDACs, although at maximal doses used (SB 8mM, TSA 1mM), some cell death was seen. At 48 hrs, MMC, 50ug/ml killed >80% of CRMM-1 & CRMM-2 cells.

**Conclusion** These observations suggest that HDACs do not affect melanocyte and fibroblast viability at doses which can inhibit growth and survival of conjunctival melanoma cells. As such, these compounds may be useful for controlling conjunctival melanoma growth, alone or combined with topical agents such as MMC.

**3363**

**In vivo GFP imaging of dormant liver metastasis in a human uveal melanoma mouse model**

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**Purpose** We previously demonstrated the feasibility of a human uveal melanoma GFP model in nude mice. We were able to monitor the preferential colonization of the liver by malignant cells during a 20-day period. In this study we extended the observation period in order to observe disease progression and metastastic growth.

**Methods** Five hundred thousand, GFP transfected, human primary uveal melanoma cells (921) were injected into the tail vein of 30 nude mice at time zero. Starting at day one, five mouse per week was imaged, using an abdominal incision to expose the liver, for up to 60 minutes before sacrifice. The liver was imaged in vivo and post mortem, while the lungs, spleen, and kidney were all examined post mortem for GFP expression.

Histopathological examination of all organs was performed to ensure that the GFP signal was arising from malignant cells.

**Results** In vivo imaging of the liver revealed positive GFP signal in all mice throughout the experiment. Malignant cells seeded the liver after injection, but there was no apparent tumor growth upon completion of the experiment. The micrometastatic foci remained viable without changes in size or intensity of the signal. No tumors were seen in any other organ at the end of the experiment. Histopathology confirmed that the cells emitting GFP were malignant cells.

**Conclusion** The results of this uveal melanoma model show great promise for understanding the previously unobservable interactions between single human uveal melanoma cells and native liver tissue. The fact that our cells successfully colonize the liver yet remain dormant for six weeks should actually mirror the disease in humans, metastases in patients takes roughly five to ten years to develop.

**3364**

**Clinicopathologic analysis in nasolacrimal canal obstruction**

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**Purpose** To analyze the histopathological results of lacrimal sac biopsies in primary acquired nasolacrimal canal obstruction.

**Methods** 150 consecutive patients who were diagnosed as primary acquired nasolacrimal canal obstruction were included in this prospective non-comparative study. Previous history of trauma and surgery to the periorbital area was accepted as exclusion criteria. Patients were evaluated for age, gender, history, and presenting symptoms. Lacrimal drainage system abnormalities were assessed. Lacrimal system irrigation, dacrocystography, and in selected cases dacryocystography was performed. All patients underwent external dacrocyctorhinostomy. Biopsy specimens were obtained from the posterior inferior flap and examined by the same pathologist.

**Results** The results of 27 male and 123 females with age ranging from 6 to 80 years (mean 47.7 ± 15.8 years) were analyzed. None of the patients had preexisting history of systemic disease. Nineteen patients had grossly abnormal or infectious appearing sac at the time of surgery. Of these, one patient had granulomatous inflammation of the lacrimal sac and was diagnosed with sarcoidosis during postoperative examination. One patient with normal appearing sac had basosquamous cell carcinoma and underwent dacryocystectomy. The remaining specimens showed chronic inflammation (n=124), fibrosis (n=19), normal mucosa (n=4), or lymphoid hyperplasia (n=1).

**Conclusion** Pathological examination of the lacrimal sac in primary nasolacrimal canal obstruction revealed chronic inflammatory changes in most of the patients. Although rare, granulomatous inflammation or tumour of the lacrimal sac might be observed in such cases. We recommend to biopsy all cases with nasolacrimal canal obstruction.
**3365**

Meningeal carcinomatosis - a hidden cause of bilateral visual loss

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**Purpose** To present an unusual case of meningeal spreading of breast carcinoma causing bilateral blindness.

**Methods** Diagnostic methods: cranial CT and MR, ophthalmic echography, perimetry, visual evoked potentials, and liquor analysis.

**Results** The 50-year-old woman experienced gradual visual loss of her left, better eye while her right eye was amblyopic. Two and a half years ago her left breast was removed due to carcinoma with concommitant radiation and chemotherapy. Bilateral optic disc oedema/hyperpension on the right eye and pale on the left eye was found with reduced visual acuity and constricted visual field on both eyes. Cranial CT and conventional MR were negative. Visual evoked responses of the right eye showed reduced amplitude of P100 wave with normal latency while it was prolonged on the left eye. Echography confirmed optic nerve oedema. First presumption was anterior ischemic optic neuropathy, but corticosteroid therapy was unsuccessful. Further deterioration and severe headaches were the indication for cerebrospinal liquor analysis. Carcinomatous cells were found and the diagnosis of meningeal carcinomatosis proven. Chemotherapy was suggested (BCN + Platinidur) in five cycles followed by Xeloda in 17 cycles. Two and a half year after the initiation of chemotherapy, it was ceased and the patient died a month later.

**Conclusion** Imaging methods alone were not sufficient to reveal the unusual way of spreading of the breast carcinoma, being the cause of bilateral blindness. It could be presumed that early chemotherapy and irradiation suppressed the carcinomatous proliferation to the other sites, but not intracranially. Survival of our patient for three years after positive liquor finding is exceptional.

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**3366**

Lacrimal gland adenocarcinoma

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**Purpose** To expose a clinical case: Lacrimal gland adenocarcinomas are tumors with epithelial origin and aggressive behavior and poor prognosis. Perineural invasion lead to diffuse infiltration of the orbit worsening the patient prognosis and usual produce pain as main symptom.

**Methods** Clinical case

**Results** A patient of 75 years was referred by an orbital inflammation resistant to three months of systemic steroids treatment. The orbital CT demonstrate a mass in the lagrimal gland with signs of periglandular bone infiltration. A lateral orbitotomy show a mass of 15 x 12 mm, classified as adenocarcinoma of lagrimal gland with affected surgical borders in the pathologic study. Complementary external radiotherapy was done. In three months the patient developed palpebral edema and pain at the orbital region. The new CT demonstrated local recurrence and the systemic extension study was negative. However seven months later the patient died due to lung metastasis.

**Conclusion** Lacrimal gland adenocarcinomas are tumors with aggressive clinical behaviour. When they develop perineural invasion, local control of the disease is difficult. In spite of the surgery and the complementary radiotherapy these tumors have a great tendency to local recurrence and systemic extension.

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**3367**

Unusual recurrent orbital tumour

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**Purpose** We present a case of an unusual CD34+ recurrent orbital tumour.

**Methods** A 62-year-old male presented with progressive left proptosis and preserved visual acuity. CT scans showed a circumscribed mass at the infronemoral orbit. After incisional biopsy, the patient preferred conservative treatment and the lesion was debulked. Further debulking was necessary in other two occasions. With radiological signs of bone invasion, left orbital exenteration was agreed in the fourth relapse.

**Results** The lid skin sparing exenteration revealed a tumour mass (45 x 29 x 27 mm) in the infronemoral orbit. There was no evidence of invasion of either the globe or the optic nerve. In all occasions, the tumour consisted of spindle cells alternating vague storiform areas with patternless areas. The tumour was very cellular, showed low mitotic count and no necrosis or ulceration. There was focal invasion of fibrous tissue, extra-ocular muscle, fat and bony fragments. The tumour was diffusely and consistently positive for CD34, S100 and vimentin. EMA and CD399 were focally positive. Several other markers were negative. This slow growing lesion with low grade histological appearance and EM suggestive of Schwannian processes was diagnosed as a CD34 positive Schwannoma on the first debulking. In the next two recurrences, experts agreed with a diagnosis of a DFSP based on the diffuse positivity for CD34. In the exenteration specimen, due to the exceptional location of the presumable DFSP, this diagnosis was disputed and after EM and further reviews the case was concluded as variant of low grade MPNST.

**Conclusion** The consistent positivity for CD34 in our case has lead to diagnostic disagreement. Only after the fourth recurrence the final diagnosis of a CD34 positive low grade PNST could be made.
**3411**
Intracorneal bevacizumab for treating corneal neovascularization in an experimental rabbit model

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**Purpose**
To evaluate the safety and efficacy of intracorneal and subconjunctival bevacizumab in treating corneal neovascularization in an experimental rabbit model.

**Methods**
Eighteen eyes of 9 animals (New Zealand female rabbits) participated in this study. For assessment of corneal neovascularization we use an alkali solution (NaOH 1 M). All rabbit eyes developed features of limbal stem cell deficiency like corneal neovascularization, persistent epithelial defects, etc... The right eye of the animal received 2 injections of bevacizumab (one under conjunctiva and another intracorneal) and left eye will receive two injections of a placebo solution (made of physiological serum) as a control group.

**Results**
One month of follow up. All control corneas were revascularized to the center. Partial regression of neovessels was observed in 7 out of nine rabbit eyes treated with bevacizumab.

**Conclusion**
On the basis of the results, we suggest that bevacizumab can be effective in reducing corneal neovascularization in this experimental model.

**3413**
Comparative analysis of donor derived, gene modified dendritic cells in keratoplasty

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**Purpose**
Dendritic cells (DCs) play an essential role during the initiation of immune responses against non self antigens. Following organ transplantation, activated donor- and recipient-derived DCs participate actively in graft rejection by sensitising recipient T cells. There is increasing evidence that immature DCs induce antigen-specific unresponsiveness to self antigens in central lymphoid tissue and in the periphery through different mechanisms.

**Methods**
In this study, we compared the immunoregulatory properties of CTLA4-Ig and vIl-10 secreting DC in a high responder corneal transplantation model (BALB/c; H-2d into C57BL/6, H-2b). Both the in vitro and in vivo immune response to this approach were monitored.

**Results**
Transduction of DC with AdCTLA4-Ig prior to LPS stimulation dramatically diminished their allostimulatory capacity in vitro, as demonstrated by an inhibition of proliferation of cocultured allogeneic T cells. Compared to controls, AdvIL-10 transduced DC also showed a significant decrease in their ability to activate T cells. Whereas adoptive transfer of CTLA4-Ig transduced DC could not prolong the mean survival time (MST) (MST - 9.7 ± 3.9 days, p = 0.045, n = 6), vIL-10 expressing DC significantly prolonged corneal allograft survival (MST = 13.4 ± 5.0 days, p = 0.019, n = 7) as compared to controls (MST - 8.3 ± 2.6 days, n = 7).

**Conclusion**
Whereas adoptive transfer of CTLA4-Ig transduced DC could not prolong the mean survival time (MST) (MST - 9.7 ± 3.9 days, p = 0.045, n = 6), vIL-10 expressing DC significantly prolonged corneal allograft survival (MST = 13.4 ± 5.0 days, p = 0.019, n = 7) as compared to controls (MST - 8.3 ± 2.6 days, n = 7).

**3414**
Amniotic membrane preservation in RPMI-1640 medium – 5 years experience

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**Purpose**
Present amniotic membrane preservation method using RPMI-1640 medium and brief analysis of performed surgeries.

**Methods**
Amniotic membrane preservation and transplantation surgeries in Vilnius University Hospital Center of eye Diseases was started at the end of 2001. Till the June 2007 amniotic membrane was prepared from 5 placenta obtained during the caesarean delivery. The essence of the preparation of the amniotic membrane was as described by H.Dua and other authors. However, because of the supply disturbances in our hospital instead of the traditional medium we used RPMI-1640 medium. Before the cutting amniotic membrane to a small pieces we put the whole membrane in RPMI-1640 medium with high concentration of antibiotics and antifungals and stored in -5°C temperature for 24 hours. After that amniotic membrane we put on paper cut to small pieces and stored in RPMI-1640 medium and glicerol in -80°C.

**Results**
Before the surgery amniotic membrane was warmed in room temperature. No damage of the structure was observed. Hystopathological slides of membrane samples were performed. Preservation medium specimens for microbiological investigation were taken. From all 5 placenta series no growth was observed. Amniotic membrane was used during 179 ocular surface surgeries.

**Conclusion**
1. RPMI-1640 medium can be successfully used for amniotic membrane preservation. 2. Before final freezing in -80°C, 24 hours in high concentration of antibiotics and antifungals can guarantee high impregnation of amniotic membrane with antimicrobial medication. 3. After preservation in RPMI-1640 medium amniotic membrane retains its appearance, clarity and physical durability.

**3415**
A model for corneal endothelium specular microscopy images

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**Purpose**
As part of an ongoing project on corneal endothelium morphometry by diffraction, a model for corneal endothelium specular microscopy (CSM) images has been developed. The simulator has been developed to realistically as possible emulate real CSM images. Development of the simulator was anticipated to be valuable in examining the impact of varying quality of CSM images on different methods of corneal endothelium morphometry.

**Methods**
The model was written with the mathematical programming language MATLAB. An earlier version of the simulator was used to find theoretical functional relationships between cell density and cell size variation of the corneal endothelium and identifiable characteristics in the diffraction pattern, simulated using a numerical Fourier transform. Further development has added realism to the created images as well as ways of altering illumination profiles, image noise and contrast.

**Results**
Currently, the simulation model allows creation of realistic clinical CSM images and variation of CSM image size, cell size and density, regularity of cell shape, variation in cell size, frequency of illumination profile, weight of illumination profile, level of image distortion, and level of cell barrier contrast.

**Conclusion**
There are several different CSM instruments on the market providing CSM images of varying quality. There are also a number of, often instrument specific, tools for analysis of CSM images resulting in measurement variation depending on instrument, patient and operator. It is anticipated that a simulation model that allows standardized variation of important characteristics in CSM images will be an important tool for evaluation of existing tools for analysis of CSM images and for creation of new improved alternatives.
Descemet membrane endothelial keratoplasty using descemetorhexis and organ-cultured donor corneal tissue

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Purpose To report results of two surgical procedures of endothelial keratoplasty

Methods Prospective, noncomparative study. Thirty height eyes of 38 patients with 21 concomitant edema Fuchs dystrophy, 11 pseudophakia dystrophy, 4 decompensation of penetrating graft and 2 corneal edema after herpes endothelitis in all eyes; the recipient Descemet membrane was excised using descemetorhexis through a 3.2 mm corneal incision. In 17 cases endothelial transplant was harvested by lamellar manual dissection. In 21 cases separation of endothelium was made performed with viscoelastic dissection. A temporary gas bubble was used for donor tissue adherence. Preoperative and postoperative best spectacle-corrected visual acuity (BCVA), manifest refraction, astigmatism, pachymetry and endothelial cell density (ECD) were evaluated 2 and 6 months after surgery.

Results At the end of follow-up 35 grafts remained transparent and grafts were healed in good position. One eye required conversion to standard penetrating keratoplasty. At 2 months, BCVA in the group of Fuchs dystrophy is 20/40 and 20/25 at 6 months. In the group of pseudophakia keratopathy, BCVA is 20/100 and 20/63 at 2 and 6 months respectively. The average MR astigmatism was 1.3 D. The mean pachymetry is stable after 1 month at 540 μm. The average ECD at 2 months was 1500 cells/mm² and 1300 cells/mm² at 6 months.

Conclusion This procedure, with its absence of corneal surface incisions and sutures, is a safe procedure that preserves the normal corneal topography, minimizes astigmatism and corneal power changes. This technique offers considerable advantages over penetrating keratoplasty in the treatment of endothelial dysfunction.

Ultrastructure of Femtosecond Laser trephination for keratoplasty

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Purpose Femtosecond Laser are proposed as tools for trephination in keratoplasty. This novel technology is used to realize complex tissue dissection profiles that may potentially allow a reduced astigmatism, enhanced wound stability and more selective transplantation of tissue parts.

Methods Using a 160 kHz Intralase Femtosecond Laser (Intralase b660, Intralase Corp., CA) different trephination patterns were performed in ten porcine eyes. Corneoscleral specimen were subsequently cultured for five days before being processed for histological assessment. Corneal thickness before the procedure and after histological embedding was measured; the geometry of the dissection line was evaluated and the morphological characteristics of the cellular architecture along the lesion was analyzed at high magnification light microscopy.

Results Straight and bevelled trephinations were realized with equal technical ease after application of the corneal curvature. The focus pattern lines within the corneal stroma presented as straight lines of 5+/-10 μm crossing the histoarchitecture of the collagen lamellae without deflection within of the corneal stroma. Sporadic hematoxylin-eosin negative spots were suggestive of cellular debris altered by the laser spots. Cell nuclei were also found scattered within the dissection line.

Conclusion Tissue dissections for corneal grafting in complex bevelled patterns proved feasible with the Intralase-Device. Separation of corneal collagen lamellae occurred along the targeted line, leaving a linear cut without visible ablation of tissue nor discernable collateral damage to the surrounding tissue. The smoothness of the cut may allow, in clinical application, novel selective tissue transplantation, potentially minimizing interface haze.
**3421**

A system to perform quantitative fluorescein angiography

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**Purpose** To develop a system with which fluorescein angiograms may be quantitatively compared across time, patients and laboratories.

**Methods** Sets of standard solutions of the fluorescein were developed in media that mimics the physical and chemical environments of compartments of the eye, i.e., serum and vitreous, in which the measurement is taken. Images from these sets of standards were obtained at exactly the same time and under the same instrument settings as the data from the eye on the same angiogram. Computer software was developed to quantitatively analyze the angiogram that established calibration plots and converted the grey image to an absolute color scale image of the data, as well as provide quality control information as to the performance of the instrument.

**Results** The position and shape of the excitation and emission spectra of the fluorescein standards matched those of fluorescein in serum and vitreous media. The 80% reduction in quantum efficiency of fluorescein in serum compared to fluorescein in PBS at pH 7.4 was equal to fluorescein in synthetic serum and/or PBS at pH 5.5. The reduction in quantum efficiency of fluorescein in vitreous was only 3% and equal to fluorescein in PBS at pH 7.3. The resulting computer generated color scale angiogram images were directly compared across time and patients and the initial results appeared consistent and reasonable. In addition, by evaluating the calculated instrument performance parameters, e.g., linearity, dynamic range, detection threshold and Window of Analysis, a determination of the quality of the angiogram could be made.

**Conclusion** This system provides data that is independent from the instrument and operator and promises that quantitative comparisons can be made across time, patients and laboratories.

**Commercial interest**

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**3422**

Instrumentation adaptation for quantitative fluorescein angiograms

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**Purpose** To obtain quantitative fluorescein angiograms that are independent of instrument setup and operator influence.

**Methods** Sets of five standard fluorescein solutions were developed to mimic the environmental conditions of the plasma and vitreous compartments. Images from the eye fundus (after patient dye administration) and from these fluorescein solutions were simultaneously obtained at different focal planes via a beam splitter and lens system attached to an HRA scanning laser ophthalmoscope. Software was developed to calculate calibration plots and to convert the angiography grayscale images into absolute concentration image maps for both eye compartments. This methodology provided instrument calibration data for each image obtained.

**Results** A system of standards, beam splitting adaptor and software was developed which provides quantitative data on an absolute color scale derived from calibration standards which are designed to provide information that is independent of the instrument and operator. Such quantitative data would be comparable across patients, laboratories and time.

**Conclusion** This methodology provides a breakthrough in fluorescein angiography in that it allows direct comparison between different fluorescein angiograms either from the same patient over time or across patients and laboratories.

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**3423**

Recent advances in the analysis of non vascular features in retinal images

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**Purpose** To problem of detecting non vascular dark lesions (haemorrhages and microaneurysms) in retinal images for diagnostic purposes is addressed.

**Methods** The green channel of images is extracted and then normalized by compensating the luminosity and contrast drifts. The detection of clusters of dark pixels is based first on a local thresholding to identify pixels darker than the surrounding, followed by an evaluation of their spatial density, which tells how much the neighborhood of the pixel is dense of similar (dark) pixels. A further threshold is then applied to this density to identify candidate clusters of dark pixels.

**Results** A pool of 60-50 deg fundus images acquired on slafe, and then digitized with a 1178 dpi resolution, true colour, 6 images presented microaneurysms or haemorrhages. These images were chosen to evaluate the performance of the proposed method. On average, the algorithm was able to detect at least part of a lesion for 94% of the lesions present in an image. When an image presented only one lesion, so that a hit-or-miss situation may compromise the clinical evaluation, the algorithm was able to correctly identify the true candidate.

**Conclusion** A novel, high sensitivity algorithm for identifying dark lesions in retinal images is presented. It exploits both the local gray-level information for a first segmentation and the spatial density of segmented pixels to obtain a measure of a region homogeneity. The effectiveness of the algorithm has been successfully tested on six sets of images presenting dark lesions of various shapes and dimensions. This suggest that it can be well suited as first stage of a dark lesion detection system, which will be followed by a classification stage, able to reject possible false candidates.

**Commercial interest**

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**3424**

Advances in the detection of the retinal vasculature

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**Purpose** The detection and measurement of retinal vessels is discussed.

**Methods** A model based on fitting “ribbon of twin” active contour models, that sandwich the vessel edges between contours pulled together by forces, is used. The use of these twins, with a constraining force maintaining approximate vessel width, serves to avoid errors instigated by a variety of noise sources.

**Results** The method detects vessel segments with high reliability, and is able to measure the vessel width to within sub-pixel accuracy. We present results comparing the performance with reference standard measurements constructed by a number of human assessors.

**Conclusion** Retinal vascular measurements are potentially extremely useful in the diagnosis of a number of disease.
Is corneal hysteresis an additional risk factor for glaucoma?

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Purpose To assess the correlation and agreement between Goldmann Applanation Tonometer (GAT) and Ocular Response Analyzer (ORA). To investigate the corneal hysteresis (CH) and the Corneal Resistance Factor (CRF) in normal subjects and glaucomatous patients.

Methods Prospective controlled study including one eye at random of 41 normal subjects and 45 glaucoma patients with optic disc alteration and visual field defect. The IOP was determined by GAT and ten minutes later by ORA followed by pachymetry. Corneal Compensated Intraocular Pressure (ICP), Corneal Resistance Factor (CRF) and CH were considered for analysis. Pearson correlation coefficient and Bland-Altman plots were used to evaluate the correlation and the agreement between the two devices.

Results The mean CCT was 558±15 µm in normal subjects and 540±38 µm in glaucoma patients. The mean IOP was 17.0±2.5 mmHg for normals and 17.8±6.5 mmHg for glaucoma group. The mean IOPc was 14.2±3.9 mmHg in normal subjects and 17.5±6.3 mmHg in glaucoma. The GAT and IOPc values were correlated in both group (p<0.01). There was a significant correlation between CCT and CH in all patients (p<0.05). CH and CRF values were significantly lower in glaucoma group (p<0.05).

Conclusion The CH and CRF values are significantly lower in glaucoma patients comparing to normal subjects.

Central corneal thickness and glaucoma in adult chinese.

The Beijing eye study

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Purpose To evaluate whether glaucoma is related to central corneal thickness (CCT).

Methods The Beijing Eye Study 2006 is a population based study including 3251 (73.3%) subjects (aged 45 years) out of 4419 subjects who participated in the survey 2001 and who returned for re-examination.

Results Measurements of CCT were available for 3100 (95.4%) subjects. Glaucomatous optic nerve damage was detected in 77 (2.5%) subjects ("Optic Disc Glaucoma"), and 33 (1.1%) subjects showed "perimetric glaucoma". CCT did not vary significantly (P=0.10) between "Optic Disc Glaucoma" (551±35±4µm), "Perimetric Glaucoma" (547±4±2±2µm), and the normal group (556±3±3±1µm). CCT was not significantly associated with neuroretinal rim area (P=0.30) or mean visual field defect as measured by frequency doubling testmetry (P=0.06). In multiple regression analysis, CCT was significantly correlated with age (R=0.001), Odds ratios (OR): 567; 95% confidence intervals (CI): 3.44-7.90, urban region (P=0.001), OR: 4.76; 95%CI, 2.40-7.00) and Intraocular Pressure (IOP=0.001), OR: 1.5; 95%CI, 1.33-1.94, 4.69, while it was not significantly (P=0.07) associated with chronic per-angulus glaucoma. In 126 (14%) ocular hypertensive patients without glaucomatous optic disc abnormalities, mean CCT (582±33±4µm) was significantly higher than in the normal group (P<0.001) and than in the glaucoma groups (P<0.001). During follow-up from 2001 to 2006, 42 subjects showed a progression of, or development of glaucomatous abnormalities of the optic nerve head. The progressive subjects had slightly, not significantly (P=0.03; 95%CI: 27.4-101), thicker corneas (554±33µm) than the stable subjects (545±36µm).

Conclusion CCT may not be markedly different between glaucomatous eyes and normal eyes.
Free Papers: Intraocular pressure and treatment

* 3435
Deep sclerectomy in normal tension glaucoma
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**Purpose** To study the effect of deep sclerectomy (DS) on intraocular pressure (IOP) in normal tension glaucoma (NTG) patients.

**Methods** We retrospectively analysed 21 eyes of 18 consecutive NTG patients to whom DS was performed. A collagen implant was used (Aquaglue, Staar surgical, Nico, Switzerland) in 20 of 21 operations. Mitomycin-C (0.4 mg/ml for 3 minutes) was used in 20 of 21 operations. DS was combined with phacoemulsification in three eyes.

**Results** Median follow-up time at the last visit was 14 months (range 3 to 27 months). There was a significant reduction from the preoperative IOP (median, range; 16 mmHg, 11 to 22 mmHg) to the IOP at the last visit (10 mmHg, 4 to 20 mmHg; p=0.001). The median percentage of IOP change was -42% (81 to 19%). A 25% IOP reduction without medication was achieved in 15/21 eyes and 30% IOP reduction in 13/21 eyes. One year follow-up was available for 13 eyes. In these, IOP was reduced from the preoperative 17 mmHg (14 to 22 mmHg) to 10 mmHg (4 to 20 mmHg; p=0.001). The median percentage of IOP change was -42% (81 to 9%). A 25% IOP reduction without medication was achieved in 11/13 eyes and 30% IOP reduction in 10/13 eyes. Gonioscopy had been done in 8/21 eyes until last visit and in 6/13 eyes until one year. Complications included tearing of conjunctiva (n=3), nasal perforation (1), flap avulsion (1), prominent subconjunctival bleeding (1), hyphema (1), re-saturation of conjunctiva (3), dellen (1), loss of 2 Snellen lines (5), and later shunt operation (1). IOP was < 4 mmHg in 16/21 eyes on the first postoperative day, and in 2/21 at 4 weeks. None of these persisted.

**Conclusion** Despite low preoperative IOP, clinically significant IOP reduction was achieved. DS seems to be effective and safe in reducing IOP in NTG patients.

* 3436
Trabeculectomy with or without adjustable flap sutures and anterior chamber maintainer
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**Purpose** To compare the efficacy and safety of trabeculectomy with or without adjustable flap sutures and anterior chamber (AC) maintainer.

**Methods** 32 patients (64 eyes) were included in this prospective study. One eye of each patient was randomly assigned to a trabeculectomy with 2 adjustable and 2 releasable flap sutures + an AC maintainer (technique 1) while the other eye had a trabeculectomy with 2 releasable flap sutures + a viscoelastic (technique 2). The rest of the operation was similar in both eyes. Mitomycin-C was used in 50% of the patients in each group. The postoperative management was equal in both groups except for the manipulation of the adjustable sutures. The main outcome measures were the intraocular pressure (IOP) and the frequency of early postoperative complications.

**Results** 32 patients were included and had a follow-up of at least 24 months. The mean pre- and postoperative IOP at 24 months was 22.1 ± 7.6 and 13.3 ± 2 mmHg for technique 1, and 22.7 ± 6.8 and 13.3 ± 3.4 for technique 2 (p=0.18). Early postoperative complications were infrequent and comparable in both techniques.

**Conclusion** The efficacy and safety of both trabeculectomy techniques were comparable.
**3441**

**Oxidation damage repair in the lens: Thioldtransferase and Thioredoxin systems**

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**Purpose** Oxidative stress can modify damage protein thiols to protein-S-S-glutathione (PISSG) or protein-protein disulfides, leading to the molecular weight aggregation and cataract formation. The two thiol/disulfide regulatory systems of thioldtransferase (TTase) and thioerdoxin (TRs) are examined for their roles in oxidation damage repair in lens proteins/enzymes and protection of lens clarity.

**Methods** Human lens epithelial (HLE) B3 cells were examined for their resistance to oxidative stress of H2O2 by monitoring PISSG levels, glycerolaldehyde 3-phosphohydrogenase (G-3PD) and GSH peroxidase (GPx) activities among groups of control (vector), TTase /TRs overexpressed or cadmium treated (inhibitor to both) cells. Exogenous TTase or TRs were used to enrich the cellular TTase or TRs for the similar studies. TTase knockout (KO) mouse model was used to examine the effect of TTase deletion on lens clarity.

**Results** TTase or TRs transfectants showed 2-fold expression over the control. These cells displayed a faster and complete recovery of G-3PD or GPx activity and lowered PISSG formation. Cadmium suppressed G-3PD re-activation in cells exposed to H2O2. TRs showed similar ability to repair and reactivate these oxidatively damaged enzymes. Cells enriched with overexpressed TRs gene or treated with exogenous TRs induced upregulation of several oxidation defense enzymes. Lenses from TTase KO mice were less resistant to oxidative stress, and showed age-dependent increase in PISSG and accumulation of high molecular weight aggregates sooner than that of the age-matched wild type mice.

**Conclusion** TTase and TRs systems can repair oxidation damage and restore protein/enzyme functions in the lens. TTase is essential to lens clarity.

**Commercial interest**

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**3442**

**Redox regulation of the proteasome in human lens epithelial cells and intact mouse lens**

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**Purpose** This study examined the proteasome susceptibility to oxidative stress and changes in the redox system after proteasome inhibition.

**Results** Oxidative stress by H2O2 significantly decreased all three main peptidease activities of the proteasome, the chymotrypsin-like, the trypsin-like and the peptidylglutamyl peptidase activities of the proteasome in human lens epithelial cells (HLEC). Oxidatively stressed mouse lenses showed trends of decreased chymotrypsin-like proteasome activity as well as decreased GSH-levels in HLEC lysate, the chymotrypsin-like and the peptidylglutamyl peptidase activities were stimulated by GSH and all three peptidase activities were inhibited by GS(GSH) addition. Inhibition of the proteasome in HLEC by MG-132 and lactacystin caused increased apoptosis as revealed by Hoechst 33342 staining and caspase-3-determination. Proteasome inhibition increased superoxide and peroxide production, depolarised the mitochondria and decreased the level of glutathione. However apoptosis could be prevented by addition of GSH. Non-stressed HLEC exposed to GSH, did not present any change in apoptotic response.

**Conclusion** The present data indicate that the proteasome may regulate apoptosis through oxidative mechanisms.

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**3443**

**Enhanced diabetic cataract in mice lacking Cu-Zn superoxide dismutase**

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**Purpose** We have previously shown that lenses from mice lacking the antioxidant enzyme copper-zinc superoxide dismutase (SOD1 null mice) develop cataract and show signs of cell damage when exposed to high levels of glucose in vitro. The superoxide radical, elevated in the SOD1 null lenses, is thought to contribute to the cataractogenesis in these lenses possibly through the reaction with nitric oxide, generating the highly toxic peroxynitrite: In this study, we aimed to evaluate the effect of diabetes mellitus on cataract formation and oxidative stress in SOD1 null mice in vivo.

**Methods** SOD1 null and wild-type mice were rendered diabetic by intraperitoneal streptozotocin injections for 5 consecutive days. Mice injected with citrate buffer were used as controls. After eight weeks of diabetes, the mice were sacrificed and the lenses removed and photographed in retro-illumination. The cataract formation was quantified from the photographs using digital image analysis. Lens cell damage was assessed by analyzing the leakage of LDH from the lenses and markers of oxidative stress, such as glutathione (GSSG/GSH) and protein oxidation were measured in lens homogenates.

The results were statistically analyzed using a general linear model.

**Results** There was a strong relationship between glucose levels and cataract development, especially in the SOD1 null mice (p=0.003). The lenses showing increased cell damage also developed more cataract, an effect which also was more evident in the SOD1 null lenses (p=0.002). Also, with increasing markers of oxidative damage, the lenses showed increased cataract development (p=0.01).

**Conclusion** The study suggests that increased formation of superoxide radicals and derived oxidants might be the cause of diabetic cataract.

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**3444**

**Oxidative inactivation of the proteasome and its consequences in lens biology**

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**ABSTRACT NOT PROVIDED**
Reduced and oxidized glutathione and TT-ase activity in mouse lens after in vivo exposure to ultraviolet radiation

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Dose-response relationship for alpha tocopherol associated protection of ultraviolet radiation induced cataract

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**3451**

**Strategies for improving intra-individual reproducibility in laser Doppler flowmetry measurements**

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**Purpose** Scattering of blood flow data as assessed with laser Doppler flowmetry (LDF) in humans is a problem in many studies using this technique: We set out to reduce variability in LDF data by partitioning out the effect of the total returning light level (DC) on LDF parameters in the choroid.

**Methods** In 20 healthy subjects choroidal blood flow was measured at different DC values using a portable confocal LDF device. To reduce scattering of data partitioning out the effect of yield defined as DC gain, firstly a previously described method based on a third order polynomial fit using all obtained data was applied. Secondly a new method based on a linear fit obtained for each subject individually was used. In addition, these correction procedures were applied retrospectively to the data of a recent study investigating the effect of a nitric oxide (NO) synthase inhibitor on choroidal and optic nerve head blood flow parameters.

**Results** Both methods were successful in reducing scattering of LDF parameters in the choroid with varying baseline DC values. In addition, both methods minimized scattering of the effect of the NO synthase inhibitor on ocular blood flow parameters as analyzed retrospectively. Whereas, however, the previous approach altered the magnitude of the mean change induced by the NO synthase inhibitor to higher values, the new method preserved the originally reported effect.

**Conclusion** When systematic changes in DC occur after an intervention one needs to be careful in interpreting the obtained data. The approach presented here may represent an effective, easily applicable and valid way to reduce scattering of data from using LDF to assess blood flow in the posterior pole of the human eye.

**3453**

**Effects of moxaverine and placebo on ocular blood flow**

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**Purpose** A number of eye diseases are associated with perfusion abnormalities. Moxaverine, because of its known direct vasodilator effect, is used in the therapy of perfusion abnormalities in the brain, the heart and the extremities. The present study aimed to investigate whether moxaverine affects ocular blood flow.

**Methods** 16 healthy volunteers were studied in this randomized, double masked, placebo-controlled two-way crossover study. Moxaverine (Ursapharm, Saarbrücken, Germany; 150 mg) was administered intravenously over 30 minutes. Ocular hemodynamic parameters were measured before and up to 90 minutes after drug administration. Retinal arteriovenous diameters were measured with a Retinal Vessel Analyzer. Retinal blood velocity (RBV) was assessed with laser Doppler velocimetry, choroidal (CF) and optic nerve head blood flow (ONF) were measured with laser Doppler flowmetry.

**Results** RBV and ONF tended to increase by 13.6±13.3% and 11.8±12.7% respectively after administration of moxaverine, but this effect was not significant versus placebo. Moxaverine increased significantly CF by 22.6±27.9%. In retinal veins moxaverine induced vasodilatation (26.0±22.8%), but this effect was not significant versus placebo. Retinal blood flow increased after infusion of moxaverine (19.6±16.5%), but again this effect did not reach the level of significance most likely due to the small numbers of subjects.

**Conclusion** The present study indicates an increase in ocular blood flow after systemic infusion of a single dose of moxaverine in healthy subject. Further studies are warranted to investigate whether this effect can also be seen after repeated dose treatment in patients with ocular vascular disease.

**Commercial interest**

**3452**

**Effect of Travoprost and Brinzolamide on ocular hemodynamics**

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**Purpose** To investigate the effect of topical travoprost and brinzolamide on retrobulbar flow velocities and ocular pulse amplitude in glaucoma patients.

**Methods** Twenty patients with glaucoma underwent color doppler imaging of the central retinal, temporal and nasal short posterior ciliary and ophthalmic artery, as well as Pulsatile dynamic contour tonometry and ocular pulse amplitude (OPA) measurement at baseline without treatment (timepoint 1, T1), after 1 month of travoprost (T2) and subsequently after 3 months of travoprost and brinzolamide (T3). A multivariate model was used to evaluate the changes in retrobulbar flow velocities and OPA.

**Results** In the temporal posterior ciliary artery, the flow velocities remained unchanged after administration of travoprost (7.9 at T1 versus 7.7 cm/s at T2, p=0.55 and 2.6 versus 2.7 cm/s, p=0.36 for peak systolic and end diastolic velocity respectively). Peak systolic velocity significantly increased after addition of brinzolamide compared to travoprost alone (8.7 cm/s at T3, p=0.003), which was a borderline increase as compared to baseline (p=0.05), whereas end diastolic velocity significantly increased as compared to baseline (2.9 cm/s, p=0.001). Ocular pulse amplitude did not change after administration of travoprost (p=0.19), but decreased markedly after addition of brinzolamide (p=0.005) despite a more important intra-ocular pressure reduction with travoprost than after addition of brinzolamide.

**Conclusion** Flow velocities improved only in the temporal posterior ciliary artery after topical treatment with travoprost and brinzolamide in glaucoma patients. Ocular pulse amplitude decreased significantly with brinzolamide added to travoprost, but not with travoprost alone.

**3454**

**Patients with coronary heart disease show reduced dynamic retinal arterial reaction**

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**Purpose** Coronary heart disease (CHD) and related vascular disorders are able to change vascular endothelial function. Whether the dynamic reaction to luminescence flicker stimulation in retinal branch arteries and veins differs between healthy volunteers and patients suffering from CHD is investigated using the Dynamic Vessel Analyzer (DVA).

**Methods** Retinal vessel reactions to flicker stimulation were examined in 21 CHD-patients (57±9.6 years old) and in 21 age and gender matched healthy volunteers. Vessel diameters of retinal vessel segments were assessed with DVA. After baseline assessment for 1 min a monochromatic (530-600nm) rectangular luminescence flicker of 12.5 Hz was applied for 20 s for 3 consecutive times.

**Results** In most volunteers a retinal arterial dilation in comparison to the baseline as well as an ensuing reactive arterial constriction were observed. In detail we found arterial reactions in % to baseline: ---------------CHD...control. ρ value/U-test/mean maximal arterial dilation/or...2.4±1.3% 3.9±2.3 .002mean maximal venous dilation/or...4.1±2.1 4.5±1.7 .008mean maximal arterial constriction...1.0±1.2 1.3±1.3 .07

**Conclusion** Functional retinal vessel arterial reaction to flicker stimulation differs between CHD patients and healthy subjects of the same age and gender. Maximal dilatation as a response to the stimulus is not reached. This might be explained with a different basic vascular tonus as well as with possible endothelial dysfunction in CHD. Application of luminescence monochromatic flicker stimulus to retinal vessels may represent a possible method to assess the state of impairment of regulatory reserve of vessels of the central circulation.
Oxygen gradients in the anterior segment of the eye

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Purpose To investigate the oxygen gradients in the anterior segment of the eye in anesthetized rabbits.

Methods Mean arterial pressure (MAP) and intraocular pressure (IOP) were measured by direct cannulation of the central ear artery and the vitreous respectively. Laser Doppler flowmetry was used to measure ciliary blood flow (CiBF; n=5), oxygen tension was measured with two fluorescent probes at the corneal endothelium and the anterior surface of the lens. To change oxygen gradients the cornea was exposed to either nitrogen or room air.

Results Baseline values: MAP 75.6±1.3 mmHg, IOP 15±1.5 mmHg, CiBF 53.8±4.2 P.U., oral mucosal oxygen saturation 89.9±0.7 %, pO2 Lens 208±3.3 mmHg, pO2 corneal endothelium 49±1.5 mmHg. Exposing the cornea to 100% N2 did not change MAP, IOP, CiBF or arterial O2 saturation. However, pO2 at the endothelial side of the cornea and the anterior lens surface dropped by 87% and 57% respectively (p<0.05). Topical brimonidine 0.2% was used to lower CiBF, which caused a significant reduction of oxygen tension in the posterior chamber by 34% and at the corneal endothelium by 8%.

Conclusion Changes of oxygen gradients in response to all perturbations were observed. The cornea is an important source of oxygen in the anterior chamber; however, changing ciliary blood flow also impacts the oxygen levels at the cornea. Oxygen gradients in the anterior segment are very sensitive to local perturbations.

Retinal oxygen saturation in humans in light and dark

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Purpose To investigate the effect of light and dark on the retinal vessel oxygen saturation (SatO2) in healthy humans.

Methods The oximeter consists of a fundus camera, a beam splitter, a digital camera and software, which calculates the SatO2 of the retinal vessels. In the first experiment, 18 healthy individuals were first placed in the dark for 30 minutes, then alternatingly in light (800cd/m2) and dark. Each light or dark period lasted for 5 minutes. In the second experiment, 23 volunteers were measured. The volunteers were placed in the dark for 30 minutes and then in light successively at 1, 10 and 100 cd/m2, each period lasting 5 minutes. Finally, the volunteers were adapted to dark for 5 minutes. Oximetry was performed at the end of each period in both experiments. Three individuals were excluded from the first experiment and 4 from the second because of poor image quality. Paired t-tests were used for analysis.

Results In the first experiment, arterial SatO2 was 3-4% higher in the dark than after subsequent adaptation to 800cd/m2 light (p<0.05) and similar results were seen in veins; 3-7% higher SatO2 values in the dark (p<0.05). In the second experiment, arterial SatO2 was 3% higher in the dark than after adaptation to 1000cd/m2 (p<0.01) and the corresponding difference in veins was 5% (higher in the dark, p<0.05). SatO2 was not significantly different between dark and 1 or 10cd/m2.

Conclusion The results indicate that SatO2 is higher in dark than in light in both arterioles and veins. The higher SatO2 in dark may reflect the increased oxygen consumption in the dark, which has to be compensated for by increasing the oxygen gradient between the retinal vasculature and tissue.

Commercial interest
Exenteration: Indications, techniques, and results of treatment
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ABSTRACT NOT PROVIDED

Exenteration enucleation and evisceration: trends and dilemmas in pathology
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ABSTRACT NOT PROVIDED

Strategies to prevent anophthalmic socket contraction in retinoblastoma patients
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Purpose The combination of enucleation plus irradiation often leads to a significant orbital volume loss and severe enophthalmos in retinoblastoma patients. Our purpose is to discuss the strategies to prevent anophthalmic socket related complications.
Methods In this retrospective study we reviewed our retinoblastoma patients who underwent enucleation. We evaluated the enucleation technique; possible causes and preventive factors for anophthalmic socket contraction.
Results In our clinic, four patients developed severe anophthalmic socket contraction following retinoblastoma treatment. One of these patients had bilateral enucleation and three patients had only one eye removal. The patient who had bilateral enucleation also had external orbital irradiation. All patients had small orbital implants (12 to 14 mm in diameter) and smaller orbital volumes. One patient had severe entropion and one patient had severe entropion with granuloma formation.
Conclusion Surgical technique, adjunctive radiotherapy, implant diameter, orbital growth retardation and fat atrophy are important factors in development of anophthalmic socket contraction in retinoblastoma patients. Most of these factors are preventable with appropriate surgical approach in enucleation.

Conditions for an ideal socket
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ABSTRACT NOT PROVIDED
Retinoblastoma and the Anophthalmic socket reconstruction

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ABSTRACT NOT PROVIDED

Reconstruction of the congenital anophthalmic/cryptophthalmic socket

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Purpose We discuss considerations and the difficulties encountered in socket reconstruction of three cases of congenital anophthalmic/cryptophthalmic socket.

Methods 3 cases of congenital anophthalmic/cryptophthalmic socket underwent surgery following a period of socket expansion using prosthesis expanders. 2 cases had socket reconstruction using baseball implant and buccal mucosal membrane grafting, and the third case had socket expansion surgery by lateral orbital wall burring and buccal mucosal membrane grafting.

Results The 2 cases with baseball implants had late postoperative problems. One case had implant migration causing lower eyelid ectropion, and chronic infections. The second case had an over prominent implant causing difficulty with eyelid closure which became worse over one year. Both cases underwent further surgery with implant exchange for Dermis Fat Grafts, giving satisfactory results. The third case had an initially good result, but with growth of his head the orbit remained smaller than the other side.

Conclusion We recommend the primary use of Dermis Fat Graft in congenital anophthalmic/cryptophthalmic sockets and in our opinion baseball implants should not be used in these cases. In our experience buccal mucosal membrane grafting has only limited success in these cases.

Overview of therapeutics for improving the orbital volume in the socket syndrome

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Purpose After the disfiguring orbital procedures of enucleation or evisceration, the cosmetic results of reconstructive surgery are often unsatisfactory because of a discrepancy between the volume of the orbital cavity and that of the orbital implant used to replace the eyeball. This result in typical findings of the postenucleation socket syndrome (PESS): enophthalmos, backward tilting of the prosthesis, unsightly depression on the superior sulcus, malposition of the upper eyelid with retraction or ptosis, and stretching of the lower eyelid causes by the gravitational pull of the prosthesis.

Methods Several pathophysiologic mechanisms have been proposed to account for these findings including volume deficit related to the eye loss, orbital fat atrophy and rotary displacement of the orbital contents, contraction of orbital structures.

Results We will describe the many procedures proposed to restore the orbital volume: placement of a secondary larger implant, injection of fat, injection of hyaluronic acid, subperiostal or intra orbital implants. We will describe the advantages and disadvantages of each one.

Conclusion PESS is not a futility. It can be restore by surgery.
# 4111
Reconstruction and replacement of the ocular surface
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**Purpose** To describe a protocol for the reconstruction and replacement of the ocular surface (OS).

**Methods** Corneal epithelial stem cell deficiency (SCD) can result in a number of changes ranging from metaplastic corneal epithelium to complete corneal breakdown. Diagnosis of SCD is essentially clinical. Demonstration of goblet cells in corneal epithelium by impression cytology or biopsy is confirmatory. The extent of SCD is defined as unilateral or bilateral and partial (visual axis affected or spared) or total. Evaluation of the thickness and clarity of the corneal stroma underlying the fibrovascular pannus, the visual potential by electrophysiological tests, the intraocular pressure, work-up of the patient for immunosuppression and HLA typing of the patient and potential living related donors are also required before embarking on treatment.

**Results** For Partial SCD where the visual axis is involved, Sequential Sector Conjunctival Epithelioctomy is indicated. For total unilateral cases auto limbal transplantation is the treatment of choice and for total bilateral cases allo-limbal, cadaver or living related donor transplants are indicated. Amniotic membrane as a patch or graft and ex vivo expanded cell sheets can also be used. Associated lid abnormalities, symblepharon (buccal mucosa graft), glaucoma and cataract should ideally be dealt with prior to undertaking OS restorative surgery. When a corneal graft is needed it is best done as a second procedure after the ocular surface has been restored. When an intumescent cataract is associated with raised pressure, corneal gaiting may become a necessity if a dense fibrovascular pannus or corneal scar precludes visualization of the interior of the eye. Long term immunosuppression is usually required.

**Conclusion** This approach provides the best chance of success.

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# 4113
DLEK to DSEK to DMEK
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**Purpose** To describe the preliminary clinical results of selective transplantation of organ cultured donor Descemet membrane (DM) carrying autologous corneal endothelium through a 3.5 mm incision, tentatively named Descemet membrane endothelial keratoplasty (DMEK), for the management of corneal endothelial disorders. Design: Non-randomized clinical trial.

**Methods** In ten patients with Fuchs' endothelial dystrophy or pseudophakic bullous keratopathy, DMEK was performed. A 3.5 mm clear corneal incision was made, the anterior chamber was filled with air, and DM was stripped off from the posterior stroma. A 9.0 mm diameter DM roll was harvested from an organ cultured donor cornea-scleral rim, and inserted into a recipient anterior chamber. The donor tissue was gently unfolded, positioned onto the posterior stroma, and secured by completely filling the anterior chamber with air for 30 minutes.

**Results** At one month, six eyes had a best corrected visual acuity of 0.5 (20/40) or better, and three eyes reached 1.0 (20/20) or better. At six months, the endothelial cell density averaged 2030 ± 373 cells/mm² (n=7). Three eyes showed a complete detachment of the donor tissue in the early postoperative course, that was managed by removal of the transplant and a secondary Descemet stripping endothelial keratoplasty procedure.

**Conclusion** DMEK may have potential to become the most preferable technique to manage corneal endothelial disorders, because it provides quick and nearly complete visual rehabilitation. Since the donor tissue required can be prepared from organ cultured corneas-scleral rims, the procedure may be readily accessible to most corneal surgeons.

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# 4112
The DSAEK development and its modification for improved endothelial survival
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**Purpose** To evaluate the effect on postoperative endothelial survival of a modified DSAEK technique for the insertion of the donor tissue into the anterior chamber.

**Methods** Twenty consecutive patients undergoing DSAEK and with a minimum follow-up of 12 months are included in this study. The first 6 patients were operated on with a standard technique, including the so-called ‘taco’ manoeuvre to introduce the donor tissue folded and held with a special forceps into the anterior chamber. In the following 34 patients surgery was modified by using a crocodile vitreous forceps to ‘drag’ the donor graft into the anterior chamber in various ways. The cornea module of the HRT II system (Heidelberg, Germany) was used to perform endothelial cell counts in all patients, 12 months after surgery (3 counts were made for each examination an the results averaged). The results were analyzed statistically with an unpaired Student’s t-test.

**Results** Endothelial cell loss varied between 28% and 75% in the first group (2 grafts showed evident clinical signs of endothelial decompensation and were exchanged) and between 16% and 24% in the second group. The difference between the two groups was highly significant (p < 0.01).

**Conclusion** Endothelial cell survival after DSAEK can be substantially improved by reducing surgical manipulation at the time of insertion of the donor graft into the anterior chamber.

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# 4114
PKP versus LKP: rehabilitation speed, optical performance, endothelial cell loss
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Ghent

This presentation will provide a critical comparison between penetrating and lamellar keratoplasty as far as rehabilitation speed, optical performance, endothelial cell loss and immunological rejection rate is concerned. Deep anterior lamellar keratoplasty has the advantage of retaining a stronger globe and no risk of late endothelial failure, implying patients might be able to keep their graft for the rest of their life. There is also a reduced risk of graft rejection, or at least the damage caused will not be directed towards the vital endothelial cells. This however seems to go at the expense of visual acuity and quality. High postoperative astigmatism remains an issue and interface haze -when dissector is not down to bare Descemets- limits the vision to 20/40 in some cases. The decrease in recipient endothelial cell loss mimics the physiological cell loss of non operated corneas except maybe in patients with macular dystrophy. The results of deep endothelial keratoplasty (either DLEK of DSAEK) seem very promising as far as astigmatic control is concerned as there is virtually no astigmatic change induced. Vision outcome in the largest reported series of DLEK was a vision of 20/40 or better in 49% of eyes. This compared to a percentage of respectively 62% and 87% in patients with DSEK or DSAEK. Contrary to common belief endothelial rejection can still happen in patients undergoing deep endothelial keratoplasty. DSAEK seems to induce more endothelial cell loss than DLEK which is probably a direct consequence of the current tacofolding techniques. New insertion devices are currently developed to minimise this increased cell loss.
The role of the Femtosecond (FS) laser in lamellar keratoplasty

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Methods: A 30 kHz FS laser (IntraLase Corp, Irvine, CA) was used to prepare a lamellar cut in the cornea of human donor eyes. After FS laser preparation of a 9.5 mm wide lamellar cut at a depth of 400 micron, the corneoscleral button was stored in organ culture medium. The endothelial viability was assessed after preparation of the lamellar cut and after dissection of the donor posterior lamellar disc (PLD) from the anterior cornea. In ten eyes of ten patients with pseudophakic bullous keratopathy the PLDs were used for endothelial keratoplasty. The follow-up ranged from 13 to 26 weeks.

Results: The mean ± sd endothelial cell loss after FS preparation of the lamellar cut (n=4) was 3.4±3.5% and after dissection of the posterior lamellar disc (n=5) was 6.5±3.2%. There was no significant difference in endothelial cell loss between FS and non-FS laser treated corneas after organ culture (p=0.46). All eyes had a clear graft with functioning endothelium. In 4 eyes BCVA was 20/40 to 20/50. In the other 6 eyes BCVA ranged from 20/200 to 20/40 and 5 of these had pre-existing maculopathy. The surgically induced astigmatism was 2.1 D. Longer-term results will be presented to assess graft clarity and visual outcomes in the other eyes.

Conclusion: FS laser preparation of posterior lamellar discs is safe and feasible for performing FS-DSEK. We believe that FS-DSEK offers a great potential for facilitating endothelial keratoplasty in a more standardized, automated fashion. Longer-term follow-up will be necessary to assess final corneal graft clarity and visual outcome.
**4121**

**Fluorescein and ICG angiograms : still a gold standard**

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**Purpose**
To review the role of Fluorescein Angiography and ICG in the management of ARMD

**Methods**
Fundus Fluorescein Angiography (FFA) is the gold standard for classification of Choroidal Neovascular Membranes (CNVMs) in Age Related Macular Degeneration (ARMD). CNVMs may be defined as classic, minimally classic or occult by their filling patterns observed on angiography. The findings of the large TAP and VIP studies are based on angiographically defined CNVMs. FFA in the setting of ARMD is indicated when the patient complains of new metamorphopsia, experiences unexplained blurred vision or there is evidence of subretinal fluid, haemorrhage or hard exudates.1,2 Fluorescein Angiography is an invasive procedure and carries a risk of anaphylaxis and death. There should be on site facilities readily available to manage any potential complications of an FFA. Indocyanine Green Angiography (ICG) is used to evaluate the choroidal circulation and is of particular use in the diagnosis of Idiopathic Polypoidal Choroidal Vasculopathy.

**Results**
With the advent of new therapeutic modalities there is a need for frequent monitoring of the response of the CNVM to treatment. Ocular Coherence Topography complements FFA and ICG, reducing the number of angiograms required during the quiescent phase of treatment. In spite of this invasive angiography remains the gold standard for diagnosis and treatment

**Conclusion**
References 1 Summary benchmarks for Preferred Practice Benchmarks in the Setting of ARMD. www.aao.org. 2 International Clinical Guidelines for ARMD. www.icoph.org

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**4122**

**Reproducibility of OCT in choroidal neovascularization secondary to ARMD**

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**Purpose**
Optical coherence tomography (OCT) is commonly used to quantitatively measure macular thickness in several macular diseases, especially in conditions associated with macular vascular leakage such as diabetic macular edema (DME) and choroidal neovascularization (CNV). Several studies reported good reproducibility of commercially available Time Domain OCTs. Few studies have investigated the role of OCT in the diagnosis and management of CNV. These studies involve relatively small numbers of patients. To date no proper reproducibility studies have been conducted in exudative AMD. With the increasing importance of OCT in patient care and the common use of OCT as a key outcome measure in recent clinical trials of exudative AMD, it is important to determine the reproducibility of OCT measures in a large population of patients with CNV.

**Methods**
In the current study, we report the reproducibility of retinal thickness measurements made with the Zeiss OCT3 in 180 patients with exudative AMD. Reproducibility is assessed for retinal thickness in the central subfield, center point thickness (measured using the automatic software algorithm), and foveal thickness measured manually. Furthermore particular changes in retinal morphology, such as cystoid abnormalities, subretinal fluid, or presence of pigment epithelial detachment are analysed. These findings will be compared with the currently available literature.

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**4123**

**Phenotype-Genotype correlations : more than identifying risks ?**

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**Purpose**
To critically review current knowledge on genetic susceptibility for AMD and its relevance in a clinical setting.

**Methods**
AMD is a multifactorial disorder with both environmental and genetic risk factors. Genetic knowledge of the disease made a real breakthrough in 2005 after 3 concomitant publications incriminating complement factor H (CFH) as a susceptibility gene, confirming the major role of inflammation in the disease pathogenesis. Further studies suggested the existence of other susceptibility genes, such as the C2/Complement Factor I (CFI) or LOCS387715-PLK wealthy I (HTAIR). Identification of other susceptibility genes is expected and groups, including ours, are putting together large cohorts of patients using an unbiased genome wide scan (GWAS) approach. Discovery of new genes will generate potential molecular targets useful to develop novel therapeutic approaches. However, these new genetic findings raised different questions that will be discussed based on our experience and the current literature.

- How can we refine correlation analysis between specific susceptibility alleles, environmental factors, systemic biomarkers and specific subgroups of AMD?
- In particular, how pertinent are the current AMD classifications to outline these correlations and should new classifications, based for instance on OCT, fundus autofluorescence patterns or functional data, be generated?
- Due to the important relative risk associated with the CFH or LOCS387715 susceptibility allele and regarding the cost of genotyping approaches such as GWAS, how can we narrow down the targeted population to increase the likelihood of finding new susceptibility genes?

- Is a systematic genotyping strategy for CFH and LOCS387715 of any AMD patient relevant in a clinical setting?

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**4124**

**Autofluorescence : additional value in clinical management of ARMD**

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**Purpose**
Autofluorescence is a noninvasive technique that allows the assessment of the viability of the RPE/photorceptor complex. With the higher expectations of newer biological treatments in age related macular degeneration (ARMD), the assessment of the viability of retina and RPE at the beginning of treatment is a precious indication on the potential therapeutic results.

**Methods**
We studied 79 eyes with untreated early-stage subfoveal neovascular AMD. Autofluorescence images were compared with digital fluorescein angiography and fundus color photographs. Autofluorescence at the macula was correlated with visual acuity, angiographic lesions characteristics, lesion size and length of symptoms.

**Results**
In 54 eyes, the autofluorescence was present, continuous at the macula and this correlated significantly with visual acuity, lesion size, length of symptoms, but not choroidal neovascularization type.

**Conclusion**
Intact autofluorescence at the macula correlating with visual acuity confirmed the hypothesis that visual outcome is determined by maintenance of RPE function. Autofluorescence assessment may allow a better identification of patients who would benefit substantially from ARMD treatment.
**Microperimetry: understanding what went wrong or predicting therapeutic routes**

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**Purpose** To investigate macular sensitivity and fixation patterns with automatic fundus perimetry (microperimetry) in patients with early and late age-related macular degeneration (AMD).

**Methods** A hundred and twenty eyes of 101 consecutive patients with early AMD or with subfoveal choroidal neovascularization (CNV) secondary to AMD were evaluated. Best corrected visual acuity (ETDRS charts), fundus photography, and fluorescein angiography were performed. Microperimetry (MPI, Nidek Technologies, Padova, Italy) was used to quantify macular sensitivity and fixation patterns (location and stability).

**Results** Macular sensitivity was significantly decreased over large drusen and over pigment abnormalities (p<0.0001) and in patients with CNV when compared to normal age-related values (p<0.0001). Fixation moved from central to eccentric and from stable to unstable in patients with active CNV (p<0.01) and p<0.05 respectively. Angiographic classification of subfoveal CNV (occult versus classic) was not significantly related to fixation pattern (p<0.05). In cases that responded to treatment retinal sensitivity and fixation characteristics improved significantly (p<0.05).

**Conclusion** Microperimetric quantification of macular sensitivity and fixation patterns in eyes with early and late AMD offers new data about the impact of visual impairment in these eyes. In retinal areas affected by early AMD retinal sensitivity deteriorates, despite good visual acuity. Microperimetry may allow early detection of functional impairment due to these lesions. Moreover, microperimetry may be of value in predicting the outcome of CNV when different treatments (laser, PDT, anti-VEGF drugs, macular translocation and RPE transplants) are planned in current or future studies.

**4126**

(Multifocal) ERG: additional functional information in research and clinical settings

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**Purpose** To investigate the relationship between areas of hyper- and hypoautofluorescence and measures of visual function in patients with macular diseases.

**Methods** Eight patients (8 eyes) with macular disease aged from 26-80 yrs. were enrolled in the study. All tested eyes had best corrected visual acuities of at least 20/100 and areas of abnormal fundus autofluorescence (FAF) in the posterior pole. Retinal function was evaluated with the multifocal ERG (mfERG) VERIS system using a display of 61 hexagons. Fixation was continuously monitored. First order kernel responses were analyzed.

**Results** The pattern of hyperautofluorescence varied with the macular findings as well as the stage of disease. For all patients, MP-1 and HVF fields showed decreased sensitivity in locations corresponding to and adjacent to hyperautofluorescent (atrophic) areas. Multifocal ERG response amplitudes were decreased and sometimes delayed in the same regions. Deficits extended into areas of normal FAF. Hyperfluorescent areas adjacent to normal appearing areas had normal visual function, whereas those adjacent to atrophic areas had decreased visual function. In patients with ABCA4 related maculopathy, flecks adjacent to normal FAF had normal visual function with the MP-1. In contrast, flecks adjacent to atrophic areas had reduced visual sensitivity. Two patients with ABCA4 dystrophies had eccentric PRLs in superior retina.

**Conclusion** The correlation of fundus hyperautofluorescence with cone system function is related to the health of adjacent retinal pigment epithelium. There is a need to use functional techniques as well as FAF in monitoring macular disease progression.
**4131**

**SITA-SWAP in glaucoma diagnosis**

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**Purpose**
The aim of the study is to verify the characteristics of the new algorithm SITA-SWAP in glaucoma diagnosis.

**Methods**
25 consecutive patients followed by the glaucoma service of the University Eye Clinic, Fondazione Policiano San Matteo, were selected as glaucoma suspects on the basis of the outcomes of standard automated perimetry (SAP), Scanning Laser Polarimetry and Confocal Scanning Laser Ophthalmoscopy. Test duration and global indices MD (mean defect) and PSD (pattern standard deviation) of 24 2 SITA-SWAP were compared to the same data from 24 2 SITA SAP (SS) and 24 2 Full Threshold-SWAP (FS). A questionnaire about individual tolerability, perception of test duration and fatigue connected to the different perimetric exams, was also submitted to each patient.

**Results**
SITA-SWAP mean test duration was 3.56 ± 0.56, significantly less than FS (14.71 ± 1.12) and SS (5.03 ± 0.41).The mean MD for SITA-SWAP was -3.42 ± 4.3, significantly different from SS (-1.31 ± 1.4) but not from FS (-2.27 ± 4.5), indicating the influence ofJon teracities on blue light transmission. The mean PSD for SITA-SWAP was 1.33 ± 1.02, significantly different from SS (1.70 ± 0.37) and from CPSD provided by FS (2.25 ± 1.17), showing a promising sensitivity of SITA-SWAP in pointing out localized defects. SITA-SWAP was evaluated as shorter, easier to perform, better tolerated as compared to FS and shorter but more challenging as compared to SS by most of the patients.

**Conclusion**
SITA-SWAP seems to be a rapid and easy perimetric test, able to help physician in glaucoma diagnosis. The blue light transmission through optic media is constantly influenced by their opacities.

**4132**

**Accuracy of scanning laser polarimetry, scanning laser tomography in a glaucoma screening trial**

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**Purpose**
To compare the usefulness of scanning laser polarimetry (GDx-VCC), scanning laser tomography (HRT) and their combined evaluation in glaucoma screening.

**Methods**
In a non-population-based pre-publicized trial, self-recruited Caucasian participants were screened for glaucoma with GDx-VCC, HRT, and by independent clinical examination. Cases with possible glaucoma as found with any of the screening methods underwent a detailed clinical investigation to verify or exclude glaucoma.

**Results**
Of the 136 attendees 118 participants (218 eyes) successfully underwent the GDx-VCC and HRT II measurements. Twenty-three eyes of 13 participants had glaucoma. Seventeen glaucomatous eyes had early damage. Evaluated separately, the GDx-VCC screening test (borderline-normal), performed best with 7.5 positive likelihood ratio (PLR). PLR for all HRT parameters was <3. Combining different threshold criteria, for GDx-VCC PLR increased to 14.0-17.7, but no useful increase was seen for HRT (PLR<4.7 for all combinations). Combination of the best HRT and GDx-VCC criteria resulted in PLR increase compared to the HRT combinations, but PLR decrease compared to the GDx-VCC combinations (PLR<12.7 for all combinations).

**Conclusion**
In this population with relatively high risk for mild glaucomatous damage, a combination of different GDx-VCC criteria but not HRT criteria or combinations containing HRT criteria were useful for glaucoma screening.

**Commercial interest**

**4133**

**Cup reversal protects from glaucoma progression**

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**Purpose**
Reversal of optic disc cupping after reduction in intraocular pressure (IOP) is well documented. The effect of cup reversal on the risk of progression of glaucoma was investigated.

**Methods**
All 80 consecutive patients referred to the Helsinki University Eye Hospital between May 1995 and May 1997 because of exfoliation glaucoma were included. They were treated medically, with laser trabeculoplasty, or surgically. Fifty-six patients (mean age <67 at study entry, 70 ± 5 years) were followed for 5.9 ± 0.8 years. Scanning laser ophthalmoscopy (Heidelberg Retina Tomograph, HRT) of the optic nerve head was performed before treatment and at follow up visits. A decrease of more than 5% in the HRT parameter cup-volume (CV) after change in treatment was considered to be a sign of cup reversal. The effect of cup reversal on progression of glaucoma was modelled in multiple logistic regression.

**Results**
Of 56 patients, 24 showed progression of glaucoma in visual fields or optic discs. The other 32 remained stable. IOP reduced from 21 ± 5 mmHg to 16 ± 4 mmHg (P <0.001). Presence of cup reversal was related to decreased risk of progression of glaucoma (Oddsratio[OR], 0.226; 95%CI, 0.055-0.917; P = 0.037). The risk of progression increased with higher MD at study entry (OR, 1.158 for each dB; 95% CI, 1.034-1.296; P = 0.011), and higher final IOP (OR, 1.216 for each mmHg, 95% CI, 1.000-1.479; P = 0.050). The change in IOP from study entry to final IOP was not associated with progression progression (OR, 0.964 for each mmHg, 95% CI, 0.850-1.092, P = 0.561).

**Conclusion**
Optic disc cupping can be reversed and sustained for years after IOP reduction. Cup reversal seemed to be an independent protective factor against progression of glaucoma.

**4134**

**Combination of structural and functional methods for the diagnosis and follow-up of glaucoma: results of a two-year study**

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**Purpose**
To determine, whether an appropriate combination of the structural and the functional examination methods can improve the diagnosis and follow-up of glaucoma.

**Methods**
One randomly selected eye of 40 patients with primary open angle glaucoma (POAG) with none or early glaucomatous visual field loss and 40 age matched healthy persons were included in the prospective longitudinal study. Structural evaluations were conducted using Heidelberg Retina Tomograph (HRT) and retinal nerve fiber layer (RNFL) loss scoring according to Airalainen method. Functional parameters were reviewed by means of standard white-on-white (W/W) and blue-on-yellow (B/Y) perimetry. To assess the diagnostic value of different data sets machine learning classifiers , linear discriminant analysis and classification trees were applied. The accuracy of discrimination was described and visualized by the Receiver Operating Characteristic Curves (ROC), and the results of the first and second year of study were compared.

**Results**
There were statistically significant differences between the healthy and glaucomatous group in the scoring of RNFL loss, HRT parameters (C, C/D, RRD, RVS, CSM) and mean defect of W/W perimetry. Parameters with the highest diagnostic ability obtained by ROC curves were scoring of RNFL loss, HRT analysis of the optic nerve head and W/W perimetry.

**Conclusion**
Combination of different diagnostic methods can enhance precision of early diagnosis and follow-up of glaucoma. A limitation for a relationship between structure and function is the individual variability of the optic disc morphology and subjective variability of visual field testing. Supported by IGA NR 8371
**4135**

Morphologic factors associated with progressive glaucomatous optic disc damage in patients with primary open angle glaucoma and low tension glaucoma

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**Purpose**

To investigate the influence of morphologic characteristics on the rate of progression in patients with open angle glaucoma with and without elevated intraocular pressure.

**Methods**

One hundred eight eyes of 54 patients with low tension glaucoma and 128 eyes of 64 patients with open angle glaucoma were recruited from the Erlangen Glaucoma Registry for this study. All included patients received an annual standardized glaucoma examination. Progressive glaucomatous optic disc atrophy was determined as rate of neuroretinal rim loss by masked comparative analysis of stereographic optic disc slides by two experienced observers independently. Standard parameter from Scanning Laser Tomography (HRT I, Heidelberg Engineering) from baseline examination were compared between stable and progressive patients in both groups.

**Results**

Neuroretinal rim area and volume was already at baseline significantly lower in the progressive group in patients with open angle glaucoma (1.26 +/- 0.38 mm^2 vs. 1.57 +/- 0.48 mm^2; p=0.001 and 0.28 +/- 0.17 mm^3 vs. 0.41 +/- 0.23 mm^3; p=0.001, Mann-Whitney U test) but not in patients with low tension glaucoma (1.07 +/- 0.39 mm^2 vs. 1.16 +/- 0.40 mm^2; p=0.404, and 0.19 +/- 0.12 mm^3 vs. 0.25 +/- 0.15 mm^3; p=0.180, Mann-Whitney U test) if compared with the non-progressive group.

**Conclusion**

Patients with open angle glaucoma with and without elevated intraocular pressure seem to differ concerning morphologic factors for eventual progression. Low neuroretinal rim area or volume as quantified by Scanning Laser Tomography may be a risk factor for further neuroretinal rim loss in patients with open angle glaucoma.

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**4136 / 412**

Structure–function relationship in the process of primary open-angle glaucoma - an OCT study

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**Purpose**

To evaluate the strength and pattern of the relationship between visual field (VF) sensitivity and retinal nerve fiber layer (RNFL) thickness as measured by StratusOCT, and define the structural decay where glaucomatous VF defects appear.

**Methods**

Normal, preperimetric (PPG) and glaucomatous (POAG) subjects were enrolled in this cross-sectional study. Linear and non-linear regression models were used to define the relationship between average RNFL thickness (AVG) and VF sensitivity (mean deviation-MD and mean sensitivity-MS) on standard automated perimetry, coefficient of determination (R²) was calculated, their association was described by bivariate Pearson correlation.

**Results**

The correlation of AVG and MD/MS was significant only in POAG eyes (r 0.718/0.733), their relationship fit a curvilinear regression model (R² 0.723/0.694), in normal and PPG groups no correlation and a minimal degree of determination was detected. Receiver operating characteristic (ROC) curves describing the ability of all VF parameters and AVGs were evaluated to differentiate PPG from POAG eyes. Repeated analysis with the best performing test parameter, PSD (AUROC=0.957) with cutoff value 1.9 dB, showed that regression profiles only in the POAG group with PSD >1.9 dB maintained their strong curvilinear RNFL/VF relationship.

**Conclusion**

Evaluating structure/function relationship in our normal, PPG and POAG subjects, strong curvilinear regression was found in POAG eyes with PSD >1.9 dB and RNFL AVG thickness below 70 µm, which might represent a profound threshold value in glaucomatous structural changes, while no correlation was detectable above these values. This is comparable with histologic data, indicating that 25-40% of RNFL is lost before VF defects arise.
**SIS: Ocular straylight measurement**

### 4141
**Basics of straylight in the human eye**

**VAN DEN BERG TJTP**
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**Purpose** Large angle light scattering in the eye causes retinal straylight and as a consequence functional disturbances such as glare hindrance while driving at night, or contrast loss in counter light situations. Apart from small angle effects such as measured with wavefront approaches, this constitutes a second major optical deficit of the human eye. Straylight must be studied for clinical use.

**Methods** A forced choice psychophysical assessment technique was developed ("Compensation Comparison"), that resulted in a commercial product (C-Quant from Oculus). The task of the patient is to compare flicker strength in two half fields, and indicate with push buttons for 20 short presentations which half field flickers most strongly. The straylight value is obtained including a reliability estimate. Added value in comparison to visual acuity was assessed in 2400 subjects in the European GLARE study. In 150 patients straylight data were collected before and after cataract procedures or YAG capsulotomy.

**Results** Repeated measures standard deviation for the method is about 0.07 log units, to be compared with differences in the young normal population of 0.2 log units, and by 1.0 log units (a factor of ten) or more with (early) cataract or corneal disturbances. In healthy eyes a strong age effect occurs. Straylight doubles by the age of 65, and triples by the age of 77. In pseudophakia, straylight values could be very good, better even than in the non- cataract group, but with much differences in the population. In age-normal as well as cataractous eyes, visual acuity and straylight were found to vary quite independently.

**Conclusion** Straylight assessment has added clinical value. It is quite independent from visual acuity. Lens extraction potentially reverses the strong age-normal increase in straylight value.

**Commercial interest**

### 4142
**Glare as parameter of quality of sight**

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The concept of glare, as determined by the Oculus C-Quant device, is discussed in detail. Its clinical use and limitations are highlighted and correlations with other ocular parameters are investigated. As illustration the use of the C-Quant in the University Hospital Antwerp for cataract and refractive cases is given, as well as for more challenging cases (postoperative complications, perforations ...).

### 4143
**Intraocular straylight and contrast sensitivity after LASIK**

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**Purpose** To give a review about the effect of refractive surgery on glare, intraocular straylight and contrast sensitivity and to present the results of a study at the Centro de Ofthalmología Barraquer.

**Methods** Altogether 50 eyes of 33 patients (mean age 31 years, refractive error range 3.5 to -7.5 D) were treated for LASIK with Bausch & Lomb Technologies Ziemer and studied pre-op and two month post-op. Intraocular straylight was measured with the Oculus C-quant and contrast sensitivity with the CSV-1000 (at 3, 6, 12, 18 cycles/degree).

**Results** In our study at the Centro de Ofthalmología Barraquer, pre-op mean log(\(\varepsilon\)) straylight parameter of 0.98 (+/−0.06 confidence interval [CI]) did not change at two month post-op 0.97 (+/−0.05 CI). Only two eyes out of 50 had straylight values increased by more than 0.2 log units. There was no significant change in contrast sensitivity at all four spatial frequencies.

**Conclusion** This and one earlier study by Leethuizen showed no change of the mean values of intraocular straylight at one or two month post-op after refractive surgery, but a few cases had a more than 0.2 log units increase. Results with regard to contrast sensitivity are not consistent in the literature, with improvement or decrease depending on post-op time, illumination level and type of refractive procedure.

### 4144
**Straylight after cataract operation**

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**Purpose** To measure intraocular straylight in patients with pseudophakia and to compare the results with those of patients in a large population sample without eye diseases and with different signs of cataract.

**Methods** In a large prospective European study with five participating eye clinics a total of 2211 active drivers, 45 years of age and older, were included. The examination included a complete ophthalmological evaluation with multiple visual function tests and the measurement of intraocular straylight with a newly developed system. Lens opacities were classified with the LOCS III lens classification system.

**Results** Increasing density of lens opacification was significantly related to increased straylight values. This resulted in a modified behavior regarding the patients driving habits. Unexpectedly, patients with bilateral pseudophakia showed similar straylight values as those that were classified as having bilateral moderate cataract. Mean age of the pseudophakic patient group was similar to subjects with more severe forms of cataract.

**Conclusion** Following cataract surgery and implantation of a clear artificial lens, one would expect intraocular straylight to be significantly lower that in the control population with moderate cataract. This was not the case in this large series of patients, however several causes may be considered relevant for this observation: First pseudophakia related changes, such as untreated posterior capsular fibrosis and possibly IOL decentration, have to be considered. Secondly increasing straylight values with age that are not only lenticular of origin, but also a consequence of age-related pigmentary changes such as migration and thinning of uveal pigment from the iris, increasing vitreal opacities and loss of melanin within the retinal pigment epithelium itself.
Straylight induced by changes in the vitreous

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ABSTRACT NOT PROVIDED

C-quant for clinical screening

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Purpose A study was carried out on 105 patients referred for cataract surgery at the Princess Alexandra Eye Pavilion Edinburgh. The aim of the study was to a) examine criteria significant for referral in current practice b) explore the implications of a greater emphasis to QoL criteria.

Methods Three categories of variable were assessed - visual function (visual acuity, contrast sensitivity), cataract morphology (oxford grading system, stray light meter) and quality of life (QoL - vF14, Sparrow scales). Referral procedures followed current clinical practice. A follow up on 30 patients was carried out 3 months after surgery.

Results 78% of patients were referred for surgery and of these 77% were first eyes. The main discriminators for surgery in current practice for all patients and those with Snellen <6/12 were LogMar acuity, Pelli Robson contrast sensitivity, stray light, brunescence, and white scatter and QoL. Stray light measures correlated with LogMar (r=0.28, p<.01 and Pelli Robson r=.35, p=.01). Exploratory regression in both groups showed that LogMar distance acuity followed by the stray light measures were the best discriminators of current practice. Quality of life scales as criteria for surgery were more difficult to predict (70% as opposed to 83% accuracy) and showed Pelli Robson contrast sensitivity as the main predictor in conjunction with LogMar and stray light measures. Follow up at 3 months on QoL scales showed a very high proportion of patients reporting satisfaction.

Conclusion Stray light measures are found to predict current referral practice and quality of life scales. Current practice is from the patients perspective effective in delivering high post surgery satisfaction rates.
The EVI-genoret phenotype / genotype patient data base: a pan-European tool for retinal dystrophies and age related macular degeneration

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CHAKRAVARTHY U (8), MÖRKE T (9)

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Purpose Supported by EVI-Genoret (EU LSHG-CT-512036) To build a patient registry that provides comprehensive, well standardized and high quality information on the phenotype and genotype of patients with hereditary and age related retinal dystrophies. To foster international collaboration by providing easy access to this resource of uniformly phenotyped patients for researchers worldwide.

Methods Within the EU funded project EVI-Genoret standards for comprehensive phenotyping of patients with ARMD and hereditary retinal dystrophies (HRD) have been developed. These standards are implemented as electronic Case Report Form (CRF) in the patient registry. SOPs for clinical procedures assure high level of data quality. A 3 level system of datasets ensures comprehensive data acquisition and protection of sensible data. Data accessibility tags are used in order to establish and share data between collaborators. The database is SQL based and uses Java script as a platform independent user interface. Legal and ethical questions that arise in different countries are addressed by a professional project management.

Results The registry can be easily accessed by Internet after a registration process. The user interface enables simple access irrespective of the operating system, no special software must be installed. The electronic CRF guides through the examination. An intelligent system checks in real-time for plausibility of entered data, supports consequent entries and automatically hides non-relevant fields, thus facilitating time-effective work. All entries are stored based on a 3 level system. Level1 as basic catalogue of entries is accessible to all users, only after one to one cooperation agreement mandatory level2 can be accessed. So far 4 European centres (Coimbra, London, Paris, Madrid, Tübingen) and one in the US contribute to the registry with more than 2000 entries for AMD and HRD in the first year since its implementation.

Conclusion We present the first international patient registry for patients with HRD and ARMD based on widely accepted CRFs and clinical SOPs. This registry will serve as a valuable resource for clinical trials in both ARMD and HRD and for basic research in this field and is open for collaborators to join.

Electrophysiology and autofluorescence imaging in differential diagnosis of retinal and optic nerve disease

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Purpose Electrophysiological methods enable exact layer by layer diagnosis of the retina and help to answer frequent diagnostic ambiguities between macular and optic nerve diseases.

Methods New multifocal techniques enable topographic representation of the retinal function and can provide accurate diagnosis of macular diseases in early stages. Current trends with emphasis on non-invasive recording of EOG, ERG, pattern ERG, VEP and multifocal testing, with regards to conventional perimetry, micropereimetry and scanning laser ophthalmoscopy imaging including autofluorescence of the RPE will be reviewed in representative clinical cases. The importance of standardisation on the performing of all tests according to the recommendations of the International Society for Clinical Electrophysiology of Vision (ISCEV) is emphasised.

Results Global retinal function is well assessed by flash evoked ERGs, but these may be normal in macular disease. PERG and multifocal ERG in combination with autofluorescence imaging may delineate macular diseases in very early stages. In such cases, visual loss may, due to the resulting delay in VEP erroneously be attributed to optic nerve disease. On the other hand, optic nerve diseases usually do not affect multifocal ERG but can affect PERG, especially its N95 component. Combination of reduced N95 component and delay in VEP is strongly suggestive for optic nerve or ganglion cell disease in which autofluorescence imaging would usually be normal.

Conclusion By judging the cause of visual loss, combination of morphological features by RPE autofluorescence with electrophysiological and psychophysical methods usually leads to correct diagnosis.

Relationship between visual acuity, visual evoked patterned potentials done at different spatial frequencies and fundus findings in more than 100 Cerebral Visual Impairment affected subjects

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Purpose To analyze relationship between visual acuity (VA), visual evoked patterned potentials (VEP) and fundus state of the optic disk in children diagnosed by MR as cerebral visual impaired (CVI).

Methods Full clinical examinations, including Teller Acuity Cards (TAC) and/or optotype for decimal visual acuity, VEP and fundus optic disk findings , were done in 107 CVI affected infants (mean age 7.9 ± 6). TAC were administered at spatial frequencies ranging from 0.23 to 36 cycles/degrees, at a distance varying from 84 to 55 cm. VEPs were recorded at Oz, O1, O2, referenced to Fz. Five black and white checks sizes, 300x300x300x300, were presented. At least two spatial frequencies were performed depending upon the visual function. Statistical analysis: means and standard deviations were presented, correlation between VA and VEP, and association between optic disk features and VA were made.

Results Mean VA in decimial, was 3.55, ranging from 1.5 (p=25%) to 4.8 (p=75%). Percent success in TAC responses was 75. Mean VEP latencies in Oz varied from 112 (15) to 105.9 (300). Mean amplitudes varied from 18.8 (15) to 22.3 (300). VEP percent success was 77.6. A negative correlation coefficient (r) was found between VA and larger checks. A weak association was found between VA, VEP and optic disk features.

Conclusion Behavioral and objective tests correlate at different levels with fundus findings in CVI affected children, and are both useful in their follow-up.
Abstract

**A previously undescribed autosomal recessive retinal dystrophy**

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**Purpose**
To report a previously undescribed autosomal recessive retinal dystrophy

**Methods**
Seventeen patients from five families in two countries were ascertained with progressive visual loss and punctate retinal flecks. All patients received full ophthalmic examination, ERGs, PERG, EOG and fundus autofluorescence imaging, and gave DNA for mutational screening. They had previously received a variety of diagnoses, including “Goldmann-Favre syndrome,” “fundus flavomaculatus” and “unrecognised dystrophy.”

**Results**
All patients had reduced visual acuity, were hyperopic and had irregularity of the RPE reflex with widespread subretinal deposits. A maculopathy was associated with intra retinal or subretinal fluid in four cases. Autofluorescence imaging was particularly useful in demonstrating these signs. Angle-closure glaucoma was present in two cases. All patients had abnormality of both rod and cone full field ERG responses, with delay in the cone flicker ERGs. All had abnormal EOG light rise. Progression in ERG abnormality was documented in three families. The results of genetic analysis will be discussed.

**Conclusion**
A novel retinal disorder is described. The distinctive clinical and electrophysiological features enable directed mutational screening.
**4161**
Atypical chalazion
LOEFFLER K
Bonn
ABSTRACT NOT PROVIDED

**4162**
Subcutaneous upper eyelid lesion
LOEFFLER K
Bonn
ABSTRACT NOT PROVIDED

**4163**
Monitoring corneo-conjunctival epithelial tumors: the in-vivo approach
DE BELVIS V, MIDENA E
Padova
ABSTRACT NOT PROVIDED

**4164**
Flow cytometry in the diagnosis of OAL
RADIN PP, MIDENA E
Padova
ABSTRACT NOT PROVIDED
* 4165
Bilateral choroidal melanoma
NOVAK ANDREJČIC K, DRNOVŠEK-OLIP B, KRAIT A, MIDENA E
ABSTRACT NOT PROVIDED

* 4167
Topical chemotherapy of conjunctival tumors: a reappraisal
PEER J (1), MIDENA E (2)
(1) Jerusalem
(2) Padova
ABSTRACT NOT PROVIDED

* 4166
Mapping conjunctival tumors: a prospective study
DAMATO B, COUPLAND S
Liverpool
ABSTRACT NOT PROVIDED

* 4168
MLPA of extraocular tumors
COUPLAND S, DAMATO B
Liverpool
ABSTRACT NOT PROVIDED
**4169**

**MLPA of uveal melanomas**

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Liverpool

ABSTRACT NOT PROVIDED
**4211**

**Strampelli's osteo-odonto-keratoprosthesis**

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**Purpose** This presentation is meant for the keratoprosthesis session organized by Christopher Liu. The early technique for “osteodonto-keratoprosthesis” described first by Strampelli in 1963 is demonstrated converting the inclusion of an acrylic prosthesis into an “heterotopic autologous transplant” for the management of chemical and thermal burns, autoimmune disease (Stevens-Johnson, Lyell), trachoma or pemphigoid scarring, etc.

**Methods** The surgery comprises three phases. Phase 1: Superficial keratotomy, cleaning and repair of the ocular surface and the fornices and transplantation of buccal mucosa from the internal lip onto the ocular surface. Phase 2: Removal of a superior canine surrounded by dentine and bone. After shaping an adequate lamina of tooth and bone a central trephination is performed to receive the optical PMMA cylinder which is glued into place with dental glue. Then the entire block is placed into the inferior orbital space for biointegration. Phase 3 consists in removal of the osteo-odontokeratoprosthesis from the orbital socket, dissection of the buccal mucosa, central trephination of the host cornea and fixation of the osteo-odontokeratoprosthesis into the corneo-scleral bed.

**Results** Some results and variations of the early technique are illustrated and discussed.

**Conclusion** Although the technique was primitive and complicated, Strampelli’s idea to use autologous tissue to incorporate the artificial prosthesis into the ocular surface has to be considered of great importance for the evolution of modern techniques of keratoprosthesis implantation.

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**4212**

**UK experience and evolving strategies to improve results I - clinical results**

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**Purpose** To report the long term results of osteo-odontokeratoprosthesis (OOKP) surgery in the visual rehabilitation of patients with corneal blindness from end stage inflammatory ocular surface disease.

**Methods** A non-comparative retrospective interventional case series 36 consecutive patients treated at the National OOKP referral centre in Brighton, United Kingdom, between November 1996 and March 2006. Osteo-odontokeratoprosthesis surgery performed in line with the Rome-Vienna protocol. Anatomical retention, visual acuity, retention of best attained visual acuity, visual field, complications resulting in anatomical or visual failure, de novo glaucoma rate.

**Results** A total of 36 patients, with age ranging from 19 to 87 years (mean 51 ± 19 years), were included in the analysis. The main preoperative diagnoses were Stevens Johnson syndrome (n=16, or 44%), severe thermal or chemical burns (n=6, or 17%), and mucous membrane pemphigoid (n=5, or 14%). The remainder of the cases comprised miscellaneous causes of dry eye (n=9, or 25%), which included one each of graft versus host disease, ectodermal dysplasia, ionising radiation damage, cicatrizing conjunctivitis from topical medication, trachoma, congenital trinigermal nerve hypoplasia, linear IgA disease, Sjogren’s syndrome, and nutritional deficiency. Follow-up ranged from 6 months to nine years (mean 3.86 ± 2.45 years). Anatomical retention during the entirety of the follow-up period was seen in 72% of cases. The main factor resulting in anatomical failure was resection of the OOKP lamina, which occurred in seven cases (or 19%). Predicted resection in three cases resulted in successful planned exchange of the lamina, but two cases underwent emergency removal of the OOKP, and two cases developed endophthalmitis. HIL a matched allodriffs suffered a higher rate of lamina resection. Out of the entire cohort, thirty patients (or 83%) had some improvement in vision, twenty eight (or 78%) achieved vision of 6/60 or better, and nineteen (or 53%) achieved 6/12 or better. The best-achieved vision was retained throughout the follow-up period in 61% of cases. Survival analysis suggested that the probability of retaining vision ≥6/60 five years after surgery was 53%±10%. Vision-threatening complications occurred in 9 cases (or 25%), and included endophthalmitis, retinal detachment, and glaucoma. De novo glaucoma occurred in six patients (or 24%) but was seen overall in seventeen patients (or 47%), ten of whom required surgical treatment.

**Conclusion** OOKP surgery can be restorative and lastingly in patients suffering from end stage ocular surface disease, for whom conventional corneal surgery is not possible. The main problems seen in this study were lamina resection, particularly in allodriffs, and glaucoma.

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**4213**

**UK experience and evolving strategies to improve results II - imaging**

**FRANCIS I, HEROLD J, HILL C, LJH C**

**Brighton**

**ABSTRACT NOT PROVIDED**

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**4214**

**OOKP - the Singapore Experience**

**WONG D ET AL**

**Singapore National Eye Centre, Singapore**

**Purpose** The management of severe ocular surface diseases and end-stage dry eye disorders such as Stevens Johnson syndrome, ocular cicatricial pemphigoid and chemical burns with conventional keratoplasty or keratolimbal allografting remains unsatisfactory. The Osteo-Odontokeratoprosthesis (OOKP) is a form of keratoprosthesis (artificial cornea) surgery designed to treat the most severe of these cases, and good outcomes with long-term retention have been reported. We present the moderate-term clinical results of Asian patients who have had this device implanted within our Singapore OOKP Program.

**Methods** OOKP surgery involves a radical two-stage procedure – in stage 1, an autologous canine tooth is removed, modified to receive an optical PMMA cylinder, and implanted into the cheek. The entire ocular surface is demed and replaced with a full thickness buccal mucosal graft. Stage 2 surgery, performed 2-4 months later, involves retrieval of the tooth cylinder complex and implanting it into the cornea, after lifting of the buccal mucosal flap, corneal trephination, iris and lens removal and anterior vitrectomy. Concurrent glaucoma and vitreo-retinal procedures may also be performed at either stage, if required. Indications for OOKP surgery include bilateral severe blindness, not amenable to keratoplasty or ocular surface transplantation or with previous failed attempts.

**Results** In February 2004, the Singapore National Eye Centre embarked on its OOKP program, in collaboration with the National Dental Centre. We have now performed OOKP surgery on 22 patients, with the longest follow-up being 39 months. Of 16 patients with a mean of 19.1 months follow-up (range 5-39 months), no major keratoprosthesis-related complications have occurred, with full anatomical stability and retention in all cases. 73.3% of patients attained at least 6/12 or better vision, while 90% attained 6/24 vision, and remaining patients have attained and retained their best visual potential with the exception of one patient who lost vision unrelated to the KPro device (endophthalmitis following glaucoma surgery occurred 1 year after KPro surgery).

**Conclusion** OOKP surgery appears to be a highly promising procedure which has the potential to restore excellent vision to the most severe cases of end-stage corneal disease, when all else has failed. Longer follow-up of these cases is currently underway.
Vitreoretinal management in osteo-odonto-keratoprosthesis surgery

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Purpose To review the strategies, problems and technical issues in dealing with vitreo-retinal pathology in eyes before, during and after osteo-odonto keratoprosthesis (OOKP) surgery.

Methods A retrospective review of eight consecutive cases in which vitreo-retinal intervention was required in the OOKP programme in the Singapore National Eye Centre.

Results Six eyes had retinal detachments. Three of these had limited previously untreated retinal detachments with traction, detected on b-scan ultrasonography. Repair of the retinal detachments preceded completion of the OOKP procedures although one eye required a repeat vitrectomy for a recurrent detachment with the prosthesis in place. One eye had a massive choroidal haemorrhage resulting in abortion of OOKP stage two surgery. The choroidal haemorrhage resolved, leaving a localized traction-associated retinal detachment that was repaired during repeat stage two surgery. Two eyes had prior retinal surgery for presumed detachments with silicone oil in place prior to OOKP surgery. Stage two surgery was completed with assessment and confirmation of stability of retinal pathology. Two eyes required vitreous retinal intervention for endophthalmitis. One developed infection after stage two surgery and was successfully salvaged. The other developed endophthalmitis after endoscopic cyclophotocoagulation for glaucoma 18 months after stage two surgery. This eye was lost in spite of aggressive intervention. The use of temporary keratoprostheses and vitrectomy endoscopes during surgery was necessary in most of these eyes.

Conclusion OOKP surgery presents challenges for the vitreo-retinal colleague. Assessment of the anatomical and functional integrity of the retina prior to and after OOKP surgery is hampered. An accurately interpreted b-scan ultrason examination provides the most useful information. Vitreo-retinal surgical intervention is technically demanding and requires the use of temporary keratoprostheses and endoscopes. An individualized and realistic management strategy has to be derived for patients with vitreo-retinal pathology when OOKP surgery is embarked upon.
4221

Why is geographic atrophy more common in Iceland than in other white populations.

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Purpose Studies have shown geographic atrophy to be more common in Iceland than elsewhere. Several possible causes for this are explored.

Methods We used fundus photographs and standardized grading and classification of age-related maculopathy and age-related macular degeneration to establish prevalence and 5-year incidence. We use a questionnaire regarding lifestyle and diet. We genotyped 581 Icelandic patients with advanced AMD (278 neovascular AMD, 203 GA & 100 with mixed neovascular AMD/GA). We examined CFH and closely related genes CFHRI-4 and HTRA1.

Results Reykjavik Eye Study includes 1045 participants born 1890 – 1946. Those better nourished than the average seemed to have a protective effect against late AMD. Iris colour was not associated with AMD. Smoking may confer similarly increased risk for GA and wet AMD. CFH confers similar risk for drusen, GA and wet AMD. Changes in the promoter region of HtrA serine peptidase I (HTRA1) confer slightly higher risk to wet AMD than GA though this difference is not statistically significant.

Conclusion Smoking lead to increased risk of prevalent AMD and to increased 5-year mortality. Genetic risk for wet AMD and GA appeared similar suggesting that at least in part they share the same genetic pathway. The generation under consideration consumed hardly ever vegetables containing lutein and zeaxanthin, did however have a very high intake of Omega-3 from fish and fish oil the latter with high doses of Vitamin A added. High Vitamin A intake over a lifetime may have increased the lipotocin content in the retinal pigment epithelium and lack of lutein and zeaxanthin intake over the same lifetime may have led to serious deficit and both may have increased the risk of geographic atrophy in this population.

4222

Ten-year follow-up of drusen like lesions in mesangiocapillary glomerulonephritis II

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Purpose This study aims to assess the outcome of drusen-like lesions in patients with mesangiocapillary glomerulonephritis – II (MCGN II) and to compare their progression to that of drusen in age-related maculopathy.

Methods After obtaining consent and ethics approval, 4 patients with biopsy proven MCGN II were re-examined clinically and on fluorescein angiography 10 years after their initial fundus examination. Three patients had undergone renal transplant in the past.

Results All four patients had bilateral, fairly symmetrical drusen at the posterior pole which extended to the periphery. There was no blood, fluid nor cystoid change at the macula in either eye of any patient. No patient showed signs of choroidal neovascularisation (CNV). There was no progression in retinopathy over 10 years. This is in contrast to age-related maculopathy where 53% patients develop CNV over 7 years. Renal transplant did not affect the risk of progression of retinopathy.

Conclusion We therefore suggest that factors other than drusen alone may contribute to the development of CNV.

4223

Reduced choroidal blood flow parameters play a role in the development of neovascular AMD

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Purpose The factors that trigger neovascular AMD are still not completely elucidated. We performed a study in order to investigate if reduced choroidal blood flow parameters are a risk factor for the development of neovascular AMD.

Methods We performed an observational longitudinal 3-years follow up study. 41 patients with unilateral CNV and non-exudative AMD in the study eye were included. Choroidal blood flow (CBF) and fundus pulsation amplitude (FPA) were measured using a compact laser Doppler flowmeter and a laser interferometer respectively. Patients were divided into two groups. Patients, who developed CNV were assigned group 1 and patients who did not, group 2. A non-paired t-test was used to compare choroidal hemodynamic parameters between the two study groups.

Results 17 patients developed CNV, 20 patients did not develop CNV and 4 patients were lost for follow up. Patients in group 1 (16.2 ± 3.00 AU) showed lower CBF values as compared to patients in group 2 (18.9 ± 3.74 AU; p = 0.021 between groups). Correspondingly, group 1 (3.58 ± 1.05 μm) had lower FPA values than group 2 (2.96 ± 0.71 μm; p = 0.045 between groups).

Conclusion The current study strengthens the hypothesis that choroidal hypoperfusion plays a role in the development of CNV in AMD.

4224

Local and multifocal ERG in age-related macular degeneration

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Purpose To assess the macular ERG responses in different forms of AMD with OCT-visualisation.

Methods 60 patients (120 eyes) with AMD aged 57-82 were examined. The groups were formed based on clinical examination. OCT and fluorescein angiography data. Local ERG (L-ERG) to a red and green stimulus was recorded with MRB ERG system (Russia). Multifocal ERG was recorded with RETIScan system (Roland Consult, Germany).

Results In group 1 with hard drusen (18 eyes) ERG responses were in normal range. In group 2 with soft confluent drusen (18 eyes) a wave implicit time (IT) was prolonged (p<0.005), N1 of mERG in normal range: b-wave amplitude of L-ERG to red stimulus was reduced (p<0.014), P1 of mERG reduced in fovea and parafovea (p<0.001). In group 3a (31 eyes) with focal PDE atrophy-a-wave IT of M-ERG prolonged (p<0.002), P1 reduced in fovea (p<0.04). In group 3b (16 eyes) with focal PDE atrophy and hyperpigmentation (VA 0.4-0.6) a- and b-wave reduced (p<0.001). P1 reduced in fovea and parafovea (p<0.05), N1 in foveal hexagon (p<0.04). In group 4a with CNV with CMO foveal and parafoveal b-wave amplitude reduced (p<0.025), P1 and N1 reduced (p<0.001). P1 in paramacular ring was reduced (p<0.05). In group 4b with PDE-detachment a-wave prolonged, a- and b-wave reduced, P1 and N1 reduced (p<0.001).

Conclusion The most sensitive indicators of macular dysfunction in AMD are the implicit time of a-wave to a red stimulus local ERG and P1 response density of mERG in foveal hexagon. Normal amplitude and implicit time of N1 in soft drusen and early PDE atrophy in eyes with prolonged a-wave of local ERG provides the evidence of smaller contribution of cones to N1 of fOK of mERG.
**4225**

**Human choroidal endothelial cell growth factor signalling in age-related macular degeneration**

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**Purpose** Age-related macular degeneration (AMD) is the most common cause of irreversible visual loss in elderly populations in the western world [1]. As the population ages, the number of people with AMD will increase even further with marked economic implications. Neovascularisation by human choroidal endothelial cells (HCEC) causes wet AMD, however, the mechanisms responsible are not fully understood. In order to assist in the development of future treatments of AMD, further investigation is required into the intracellular mechanisms involved in the activation of HCECs.

**Methods** HCEC were isolated and cultured in EBM2-MV medium as previously described. Cell proliferation following exposure to selected growth factors was measured using the WST-1 assay, which was validated by manual cell counting. The effect of the growth factors on angiogenesis was assessed using a double layer Matrigel technique. The signalling involved was analysed by isolating phosphotyrosine proteins and 2D gel electrophoresis.

**Results** A consistent pattern was seen between growth factors in all experiments. For example VEGF stimulated significant increases (180% and 160%) in both proliferation and angiogenesis, whilst IGF-1 did not significantly increase either.

**Conclusion** Several growth factors may have potent roles in AMD and this complicates its treatment. The identification of the specific signalling pathways involved in growth factor stimulation may allow more specifically targeted treatments of AMD in the future.

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**4226**

**Effects of bevacizumab on HUVEC migration and capillary formation**

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**Purpose** Angiogenesis is required for retinal and choroidal neovascularisation (CNV), many physiopathological causes of blindness in age-related macular degeneration, diabetic retinopathy and retinopathy of prematurity. Angiogenesis is a complex process involving endothelial cell proliferation, migration and tubule formation. Recently, anti-VEGF molecules (pegaptanib, bevacizumab and ranibizumab) have been used intraocularly to control CNV. The aim of the present study was to evaluate the short term in vitro effects of bevacizumab in human umbilical vein endothelial cells (HUVECs) migration and capillary-like structures formation.

**Methods** Cultures of HUVECs were established and treated during 24 h with bevacizumab (0.25 – 2.5mg/ml) diluted in culture medium. Controls were incubated with excipient solutions in identical volumes. HUVECs migration was evaluated using double-chamber assay. The formation of capillary-like structures was assessed in plates coated with growth factor reduced matrigel.

**Results** Although bevacizumab at 0.25 mg/ml did not affect HUVECs migration, a significant inhibition was achieved whenever cells were incubated with 2.5 mg/ml (p<0.05). The formation of capillary structures was prevented in the cultures treated with bevacizumab at both concentrations used in a dose dependent manner.

**Conclusion** These findings indicate that bevacizumab may inhibit angiogenesis by acting on different cellular mechanisms including diminished endothelial cell migration and inhibition of the formation of new capillary-like structures in a dose dependent manner.
Variability of ocular perfusion measurements in glaucoma and healthy subjects

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Purpose Variability is a major problem in ocular blood flow measurements. It is an important determinant of sample size in clinical trials and may limit clinical usefulness if it is too high. We set out to compare the short-term reproducibility of blood flow parameters assessed with scanning laser Doppler flowmetry and laser interferometric measurement of fundus pulsation between healthy young subjects and glaucoma patients.

Methods Measurements in 140 healthy male non-smoking subjects and 140 patients with primary open angle glaucoma were included in this analysis. From two consecutive measurements performed within 5 minutes the coefficient of variation in an individual was calculated for each outcome parameter. These included the fundus pulsation amplitude (FFA), the flow, volume and velocity in the rim and the cup.

Results Mean coefficients of variation were between 3.2% and 23.8% for all outcome parameters in healthy subjects and between 4.9% and 34.3% in the patients. FFA showed the highest reproducibility although individual coefficients up to 22.5% and 34.7% were observed in healthy subjects and glaucoma patients, respectively. With scanning laser Doppler flowmetry velocity was better reproducible than volume or flow. Individual coefficients of variation for flow values were as high as 87.3% in healthy subjects and 132.2% in glaucoma/open hypertensive patients.

Conclusion Our data indicate that in healthy subjects the selected techniques provide better reproducibility than in patients with ocular disease. This needs to be considered in sample size calculation for clinical trials. In addition, the high individual differences in flow parameter readings observed in some subjects limit the use of the techniques in clinical practice.

Twelve hour variability of ocular blood flow parameters in healthy subjects and patients with primary open angle glaucoma

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Purpose Ocular perfusion abnormalities have been implicated in the pathogenesis of primary open angle glaucoma (POAG). More specifically it has been reported that POAG is associated with vascular dysfunction and ischemia/reperfusion phenomena. In the present study we compared the twelve hour variability of ocular blood flow parameters in healthy subjects and patients with POAG.

Methods In 16 patients with POAG and 16 healthy age-matched controls ocular and systemic blood flow parameters and intraocular pressure was measured every 2 hours over 12 hours. Ocular hemodynamic measurements included optic nerve head blood flow and choroidal blood flow assessed with laser Doppler flowmetry; fundus pulsation amplitude assessed with laser interferometry and retrolubular blood velocities with color Doppler imaging. A repeated measures ANOVA model was used to compare variability among these measurements between the two groups of patients.

Results Most of the outcome variables showed higher fluctuations in patients with POAG than in healthy controls. These changes were not associated with ocular perfusion pressure or intraocular pressure. Changes over time were, however, correlated among the different ocular hemodynamic outcome measures in patients with POAG, but not in healthy controls.

Conclusion Patients with POAG show an abnormal diurnal fluctuation of ocular blood flow parameters. Since we observed correlations between these fluctuations as assessed with different methods this does not appear to be related to statistical errors associated with the measurement techniques. These data support the hypothesis of vascular dysfunction in POAG.

Changed retinal capillary flow in patients with disc haemorrhages

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Purpose Vascular pathogenesis in glaucoma has been recognized and optic disc haemorrhages (DH) may pre-cede nerve fiber damage in glaucoma. The change in peripapillary retinal capillary flow after resolution of DH was investigated.

Methods Included were 25 eyes of 25 patients with DH. 17 eyes had glaucoma. Peripapillary retinal capillary flow was measured with the Heidelberg Retina Flowmeter in arbitrary units (AU). The first measurement was made right after diagnosis of DH and the second six months later. Automatic full field perfusion image analyser was used for analysis. Mean flow (MF), peak systolic flow (SF), and minimum dia-stolic flow (DF) were measured, and pulsation index (PI) calculated. Flow values are reported from the whole temporal peripapillary retina (Area 1), the same horizontal level as the DH (Area 2), and from the area specifically around the DH (Area 3).

Results A significant increase in MF after resolution of DH was detected in Area 1 (median 263 AU; range 188 – 561 AU, vs. 328 AU, 199 – 462 AU; P = 0.008), and Area 2 (255 AU, 166 – 386 AU, vs. 330 AU, 198 – 646 AU; P = 0.04). The increase in MF in Area 3 was of borderline significance (342 AU, 230 – 513 AU, vs. 402 AU, 280 – 629 AU; P = 0.10), as was the increase in SF in Area 2 (234 AU, 187 – 486 AU, vs. 600 AU, 227 – 690 AU; P = 0.07). No significant changes in DF or PI were found.

Conclusion Increase in retinal capillary flow after resolution of DH suggests a generalized decrease in retinal flow at the time DH is detected, or a compensatory increase after ischemia.

Retrolubular hemodynamics in normal tension glaucoma compared to anterior ischemic optic neuropathy

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Purpose To compare flow velocities of the retrolubular vessels in chronic vascular neuropathy in normal tension glaucoma (NTG) to those of acute circulatory disorder of anterior ischemic neuropathy (AION).

Methods Fifty-two patients with NTG (mean age: 66 ± 8 y) and 50 patients with AION (mean age: 68 ± 10 y) were included in the study. Blood flow velocities (peak systolic velocity PSV and end-diastolic velocity EDV) and resistive indices of the ophthalmic artery (OA), central retinal artery (CRA) and nasal and temporal posterior ciliary arteries were measured by means of colour Doppler imaging (CDI).

Results No differences were detected for heart rate, diastolic arterial pressure and IOP between the two groups. In AION patients systolic arterial blood pressure was significantly higher compared to the NTG group (148 ± 26 mmHg vs. 131 ± 18 mmHg; p = 0.0004). CDI revealed significant differences of the resistive index of the OA (RI AION: 0.80 ± 0.06; NTG: 0.76 ± 0.07). In AION, PSV of CRA was significantly lower compared to NTG (AION: 7.2 ± 1.9 cmm/s; NTG: 8.0 ± 1.6 cmm/s).

Conclusion The chronic vascular deficiency in NTG seems to be similar to that of the acute circulatory breakdown in AION. In both vascular disorders of the optic nerve only small differences of flow velocities may be detected by CDI.
Disturbed vascular response of the central retinal artery during hypercapnia in normal tension glaucoma

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Purpose Altered blood flow regulation might contribute to the pathogenesis of glaucoma. The purpose of the study is to investigate retrobulbar flow velocities during hypercapnia in patients with normal tension glaucoma (NTG) without systemic vasospasm and healthy controls.

Methods Sixteen NTG patients (mean age 58 +/- 14 years) and sixteen control subjects (mean age 50 +/- 13 years, p=0.10) were enrolled in this clinical study. Blood flow velocities (peak systolic velocity (PSV), end-diastolic velocity (EDV)) and resistive indices (RI) of the ophthalmic (OA) and central retinal artery (CRA) were assessed using colour Doppler imaging (CDI). Blood flow velocities were measured under normocapnic and hypercapnic conditions (increasing the end-tidal PCO2 by 15%). Blood pressure, ventilation rate and oxygen saturation were monitored simultaneously.

Results During hypercapnia, the increase of the PSV (p=0.044) and EDV (p=0.037) of the CRA was significantly reduced in NTG patients compared to healthy controls. Flow velocities of the OA increased significantly in all subjects (PSV: p=0.039, EDV: p=0.003) during hypercapnia. Blood pressure, oxygen saturation and intracocular pressure changed similarly in both study groups with carbon dioxide provocation.

Conclusion Vascular response to hypercapnia was reduced in the CRA of NTG patients compared to controls. This may indicate a faulty vasodilatory response in NTG patients without vasospastic disease.

Dynamic retinal vessel reaction in patients with primary open angle glaucoma

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Purpose Impaired vascular regulation might contribute to glaucomatous damage. Whether retinal branch arteries and veins of healthy persons and primary open angle glaucoma (POAG) patients show different reactions in response to flickering light stimulation (FLS) is investigated.

Methods Retinal vessel reactions to FLS were examined in 28 POAG patients (stage L, 54.3 +/- 9.9 years old) after 4 week wash-out of eye drops and in 28 age and gender matched medically healthy volunteers. Vessel diameters of retinal vessel segments were assessed by Dynamic Vessel Analyzer (DVA). After baseline measurement (50 s) monochromatic rectangular FLS (520±600 nm, 12.5 Hz, 20 s) was applied.

Results In most subjects fast vessel dilation compared to baseline and an ensuing reactive arterial constriction were observed. In detail we found:

<table>
<thead>
<tr>
<th>POAG</th>
<th>control</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean arterial dilation at the end of FLS, %</td>
<td>3.3±2.7</td>
</tr>
<tr>
<td>time of max arterial constriction following FLSx</td>
<td>49.9±26.7</td>
</tr>
<tr>
<td>mean venous dilation at the end of FLS%</td>
<td>2.9±1.9</td>
</tr>
<tr>
<td>area under the venous curve following FLSx%</td>
<td>-1.1±16.9</td>
</tr>
</tbody>
</table>

We found statistically significant differences between the two examined groups as marked with ‘p<0.01 (U-test).

Conclusion Functional retinal arterial and venous dilation in response to FLS does not differ between POAG patients and healthy subjects. Reactive arterial constriction following the FLS appeared later and venous restoration occurred faster in POAG. These findings might be an indication for alterations in the vascular endothelium and vessel wall rigidity in POAG, leading to impaired regulation following metabolic demand.

Abnormal peripheral vascular response to occlusion provocation in normal tension glaucoma patients

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Purpose To assess peripheral vascular reactive hyperemia in response to occlusion provocation test, using two channels laser Doppler probe in patients with normal tension glaucoma (NTG) and normal subjects.

Methods 15 patients with NTG (12 women and 4 men), mean aged 58.9 and 15 control subjects (13 women and 2 men), mean aged 60.6 were subjected to an occlusion test. The experiment comprised following steps: 1) a 5-minute baseline period 2) a 2-minute occlusion of the left hand using a 15 cm wide cuff located directly over the elbow (the pressure in the cuff was 50 mm Hg higher than the systolic pressure measured on the arm 3) a 15- minute final recovery period after occlusion. Finger hyperemia was assessed by two-channels laser-Doppler flowmeter MBF-3d, Moor Instruments, Ltd., continuously during the experiment. For measurements of hyperemia two surface probes were attached to the pulp of the second finger (mean probe) and third finger (basic probe) of the left hand. The following hyperemia parameters were measured RF (rest flow), R2 (biological zero), TM1 (time to peak flow), TH1 (half-time of hyperemia), MAX (maximum of hyperemia) and hyperemia amplitude (MAX-RF). RF 100% was calculated. Kruskal-Wallis test analysis was used to test the differences between the group of patients and normal subjects for TM1, MXF1 (basic probe) and TM2, MXF2 (mean probe) parameters.

Results In NTG patients, TM1 was significantly higher compared with healthy subjects whereas MAX was significantly lower as compared to the control group.

Conclusion Occlusion provocation test elicits a different systemic hyperemia response in patients with NTG compared with healthy subjects.
Quantitative cataract measurement

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Purpose
To provide an overview of the impact of cataract on optical image transfer and the possibilities to measure cataract quantitatively.

Methods
The current paper reviews strategies for how limitations of the imaging transfer of the eye originating from the lens can be quantitatively measured.

Results
The diameter of the pupil should be standardized in comparisons of optical image transfer. In vitro, aberrations and light scattering can be measured directly as point spread function characterized by Strehl ratio. Also, the effect of strabismus, resonant absorption and aberrations can be measured directly. All, in vivo techniques require consideration of the impact of the cornea on the optical image transfer. Further, if psychophysical sensing is used, the image transfer of the total visual system depends on the optical transfer and the neurosensory transfer. Slit lamp microscopy with slit illumination, optionally improved with Scheimpflug projection, or alternatively with retroillumination provides quantitative geographic information about the distribution of emission of light scattering, but inconsistently correlates to the image transfer. Visual acuity measures high contrast resolution. Cataract causes loss of contrast sensitivity, mainly due to scattering and stray light. The effect of stray light can be sensed psychophysically. The point spread function and aberrations in the optical system can be measured with double pass technique.

Conclusion
Cataract can be measured quantitatively as reduction of optical transfer of the eye which is limited by aberrations, light scattering, stray light and resonant absorption.

Quantification of light scattering and cataract development using the Scheimpflug's principle

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Purpose
Light entering the eye is scattered in a forward and backward direction in the cornea and lens due to their cellular architecture and the high protein content. Short wave optical radiation, UV, ionizing radiation, other internal and external risk factors and aging increase light scattering especially in the lens finally leading to cataract development.

Methods
Back scattered light can be reproducibly measured with the Scheimpflug principle generating time and space resolved lens density data. Scheimpflug's principle minimizes imaging distortion, but cornea and lens themselves distort the images, so that mathematical corrections are needed for true biometrical data. Quantification of scattering data requires standardization and correction. Standardization is necessary because each recording medium has non linear components. External standards have been evaluated, e.g. Ulbricht spheres and a diacapm solution. Correction is necessary because scattering changes in any layer of the transparent tissues in the anterior eye segment affects the data from underlying tissues, meaning that an internal standard is necessary as well.

Results
Based on these correction procedures the actual amount of data is depending on the number of optical sections recorded per eye. For evaluation of normal age-related changes in light scattering and homogenous opacities like nuclear cataracts about 4 meridians are sufficient whereas asymmetric opacities like most cortical cataracts require a higher number of meridians.

Conclusion
Densitometric analysis of a large number of images including all the corrections mentioned above should be performed with custom-designed image analytical software in order to maximize the information output from the Scheimpflug recording.

CATARACT CLASSIFICATION SYSTEMS, A CRITICAL REVIEW

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Purpose
Since the 1990s, large scale cataract epidemiologic studies have been conducted worldwide by researchers from developed nations. Valuable data have resulted from these studies. However, cataract classification, the fundamental methodology for all studies, has not been closely scrutinized. This presentation reviews cataract classification of the past and present, and suggests future improvement.

Results
Past: The investigators often choose one classification system from, e.g., Oxford, Wilner, LOCS, and some others. These were proposed since the late 1980s each with a unique design. However, to compare results from different studies, the cataract classification system must be standardized. By late 1990s, the WHO has recognized this necessity, and a new system was developed in 2008 by the WHO working group. Present: Dr H Sasaki and Dr S West were the first to use the new WHO-sponsored system. Although a simplified classification system, it is highly quantitative when compared with previous systems. The new system is now beginning to be applied. Based on experience since 2000, the usefulness and characteristics of this new system will be presented. Future: In general, cataract grading agrees reasonably well with visual acuity. However, there is a specific type of lens opacification, i.e., "deterioration of vision and yet most ophthalmologists are still unfamiliar with it. Therefore, even the WHO-sponsored system will require revisions as other subtle cataract sub-types will also be detected in the future. It must be emphasized that further improvements in cataract classification, based on the investigators' cumulative experience, are certain to continue.

LENS OPACIFICATION IN ICELANDERS 50 YEARS AND OLDER, RISK FACTORS

REYKJAVIK EYE STUDY

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Purpose
To examine prevalent and 5-year incident risk factors for cortical and nuclear lens opacification in adult citizens of Reykjavik.

Methods
For baseline examination we used a random sample from the population census for citizens of Reykjavik ≥50 years, equal proportion for both gender and each year of birth. Participation rate was 76% (1045). 5 years later 88% of the survivors participated. For diagnosis of opacification we used Scheimpflug and retroilluminated images of the lenses. For statistical analysis we used logistic regression analysis.

Results
Cigarette-smoking more than 20 pack-years increased the risk for nuclear lens opacification (OR=2.52; 95% CI 1.52-4.13; p=0.001) as did pipe- or cigar smoking (OR=2.48;95% CI 1.26-5.12; p<0.05). Systemic corticosteroid use was a risk factor for cortical cataract (OR=3.70; 95% CI 1.43-9.56; p<0.05). Spending more than 4 hours/day outside on weekdays increased the risk of grade II & III cortical cataract, OR=2.91 (1.13-9.62; p<0.05). Comparing positive rate ratio of early cortical opacities in Reykjavik and Melbourne, by upper and lower lens hemispheres, the rate is about 90 for both cities. For the lower half of the lens while for the upper half of the lens thin figure is 70s for Reykjavik and 46s for Melbourne. This is in an agreement with the difference in zenith angle between the two cities. After adjusting for age, sex and smoking nuclear cataract is associated with twofold increased 5-year mortality risk. (p<0.05).

Conclusion
Modifiable risk factors include smoking, solar UV radiation and the use of cortical steroids. Nuclear lens opacification carries increased 5-year risk of mortality and may be a suitable index for projected life span.
Prevalence and risk factors of lens opacities in urban and rural chinese in Beijing

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Purpose To determine the prevalence of lens opacities in the elderly Chinese population in an urban and a rural region of Beijing.

Methods The study included 4439 subjects out of 5324 subjects invited to participate (response rate 83.4%). It was divided into a rural part (1973 (44.4%) subjects) and an urban part (2466 (55.6%) subjects). The study was limited to participants age 40 and older and the mean age was 56.2 ± 10.6 years (range, 40 – 101 years). Nuclear, cortical and posterior subcapsular lens opacities were assessed based on standardized slit lamp photographs of the lens using a modification of the grading score of the Age-Related Eye Disease Study (AREDS).

Results Of the 4439 persons examined, 4378 (98.6%) subjects (8756 eyes) provided lens data. Prevalence of any cataract surgery was 1.3% (95% confidence interval [CI] 1.0, 1.7), being statistically independent of gender (p=0.51; odds ratio [OR]: 0.86; 95%CI 1.00, 1.34), rural area versus urban region (p=0.25), and level of education (p=0.84). Prevalence of any nuclear lens opacity was 82.0% (95%CI 80.8, 83.2); of any cortical lens opacity was 10.2% (95%CI 9.4, 11.1), and of any posterior subcapsular opacity 4.3% (95%CI 3.7, 4.9). If grade “2” of nuclear lens opacity was considered to be normal, prevalence of nuclear cataract was 50.3% (95%CI: 48.8, 51.8), and the overall prevalence of any cataract was 53.1% (95%CI: 51.6, 54.6), increasing from 6.5% (95%CI: 5.2, 7.8) in those 40 to 49 years old, to 52.3% (95%CI: 47.4, 57.1) in those 50 to 59 years old, and to 97.8% (95%CI: 96.4, 99.2) in those 70 years and older (p<0.001). Frequencies of any cortical cataract and any subcapsular posterior cataract, respectively, were 10.3% (95%CI: 9.4, 11.3), and 4.3% (95%CI: 3.7, 4.9), respectively.

Conclusion Cataract is common among adult Chinese residents in Beijing with age as the most important associated factor. In view of the relatively low rate of cataract surgery performed so far, one may expect a marked increase in the number of cataract surgeries to meet the visual needs of the growing older population in China.
Disk swelling - differential diagnosis in orbital space occupying lesions

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We frequently cannot decide with certainty whether a disk with blurred margins and slight elevation represents a pathologic condition or not. If unequivocal pathologic findings are lacking, it is up to the examiner to decide how far the diagnostic procedures should be carried out. It may be necessary to obtain neurologic consultation and to perform neuroradiologic investigations, including MRI and possibly angiographic techniques. On the other hand, after weighing the cost-benefit ratio and the risk of some side effects, the investigator may rely on his clinical acumen and decide that the condition only represents a variant of the norm. Main differential diagnosis are pseudopapiledema in hyperopia, disk drusen, ocular hyptony, posterior scleritis, optic neuritis, disk edema (due to increased intracranial pressure, due to compression of the orbital part of the optic nerve), optic nerve tumours (meningioma, glioma), metastatic carcinoma, blurred disk margins without echographically demonstrable changes, congenital disk anomalies, central vein occlusion and anterior ischemic optic neuropathy.

The use of VEP in the evaluation of optic nerve lesions

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Purpose To describe the use of VEP and other electrophysiological investigations in the assessment of optic nerve function.

Methods The techniques for recording ISCEV Standard VEP, ERG and PERG will be demonstrated. The clinical application of these techniques will be illustrated using selected cases.

Results The nature of the VEP changes in various disorders of optic nerve function will be described. Particular emphasis will be placed on the ability of electrophysiology directly to assess the function of the retinal ganglion cells, and to facilitate, in most cases, the distinction between optic nerve and macular dysfunction.

Conclusion The objective data provided by VEP and other electrophysiological investigations play an important role in the management of patients with suspected optic nerve disease.

Histopathology of optic nerve tumours

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Purpose To evaluate the distribution in Denmark of histologically verified optic nerve lesions during a period of 60 years. Histological diagnosis, gender and age of patients were collected. Changes in frequency of parameters during the observation period were calculated.

Methods All optic nerve lesions reported at the Eye Pathology Institute, University of Copenhagen, during the period 1940–99 were investigated. All similar lesions reported at all pathological departments in Denmark during 1974–99 were also investigated.

Results The number of optic nerve lesions identified totalled 313 in 298 patients. The frequency of histopathological optic nerve lesions increased significantly over the last 25 years. At present, an average of 12 lesions per year is recorded. Lesions in children accounted for 42% (130). Benign tumours constituted 33% (44) of the total number of tumours in adults and 61% (82) in children. Frequencies of glioma and optic nerve sheath meningioma increased significantly during the last 25 years. Subjects presenting with optic nerve sheath meningioma exhibited a significant difference in age at presentation: the mean age of women at presentation was seen to be 48.8 years, while the mean age of men at presentation was seen to be 29.7 years. The most frequent lesion seen in children was glioma and in adults invasion from malignant uveal melanoma.

Conclusion Histologically diagnosed optic nerve lesions are rare and consist primarily of tumours. The increase in frequency of optic nerve lesions in Denmark during the last 25 years is due to an increase in the number of benign tumours.
A rare case – a diagnostic challenge

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Purpose To present a rare tumour of the optic nerve, and to present the clinical differential diagnostic aspects.

Methods Case study, clinical and histopathological presentation. A 3-year-old boy with a few weeks of nausea, orbital edema and proptosis. Imaging and clinical findings indicated optic nerve glioma. Surgical removal of the tumour and subsequent histopathological analysis were performed. Due to regrowth of remaining tumour, orbital exenteration was performed half a year later. Histopathological analysis of the exenteration material demonstrated change in tumour morphology.

Results The tumour was a medulloepithelioma with benign morphology and a MB-1 count below 5%. The regrowth demonstrated a malignant transformation with a MB-1 count close to 100%. This is the 8th documented medulloepithelioma of the optic nerve. All eight cases had the presurgical diagnosis of optic nerve glioma. All behaved malignant.

Conclusion Medulloepithelioma of the optic nerve is a rare tumour; however, the malignant course and the possibility to cure the patient, indicates that medulloepithelioma is an important differential diagnosis in optic nerve lesions.
Advances in the Type I Boston Keratoprosthesis

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Purpose To give an update on the Type I Boston keratoprosthesis (KPro).

Methods We reviewed our recent experiences with the Type I Boston KPro with attention to design modifications, use in pediatric patients, post-operative care and management of complications.

Results The use of the Type I Boston KPro has increased dramatically since 2003, when only 57 of these devices were implanted. In 2008, more than 450 Type I Boston KPros were implanted worldwide. Recent modifications include a threadless front plate stem designed to reduce trauma and maintain the health of the carrier corneal graft. The Type I Boston KPro appears to be a viable alternative to PK in children with corneal opacities and may simplify amblyopia management. Post-operative care following Boston KPro placement remains critical, with availability of preserved vancomycin reducing the risk of endophthalmitis. Management of complications including cataract formation and retinal detachment following Boston KPro will be discussed.

Conclusion The Type I Boston keratoprosthesis has shown continued acceptance among corneal surgeons throughout the world. Ongoing work at our institution aims to improve the utility of this device while minimizing complications from its use.

An update on the AlphaCor

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AlphaCor is a new generation of artificial cornea made entirely of pHEMA. It is a flexible 2 part composite device with a transparent centre and a porous skirt, which allows biointegration into surrounding host corneal tissue. As a treatment modality AlphaCor exists between traditional keratoprostheses and human donor penetrating keratoplasty. It is most commonly used in high risk corneal regrafts where there is a poor chance of human graft survival. Data has been obtained on every case of AlphaCor implanted, making it the largest series of keratoprostheses published. With this data and resultant alterations in protocol, there has been a gradual decline in the complication rate for this device. The main complications relate to corneal melting, optic depositions, and posterior inflammatory membranes. Because of the design and technique of implantation, post-operative glaucoma is uncommon. The probability of retention at one year is 80% and the visual acuity ranges from PL to 6/6 (20/20). The procedure is "reversible" if necessary as the eye is not lost. The data indicates that AlphaCor is an effective alternative to donor corneal transplantation in cases at high risk of graft failure. Excellent visual potential and a wide visual field can be obtained.

Where are we with the supra-Descemet Cornea (Keralia)?

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Purpose To assess a non-perforating supra-Descemet Cornea (Keralia) in rabbits and patients.

Methods Over 100 rabbits and 20 human cadaver eyes received a sDSC made of 4 polymers, intrastromally (-90% depth) in 59 rabbits, on denuded DM in 17 rabbits, and in a 3rd group (15 rabbits) -45 days after the application of 1% heparin and limbus surgical removal. The corneal surface was covered with a conjunctiva Tenon flap trimmed -63 days in postop. 9 last resort cases received a Keralia over the span of 5 years.

Results sDSC implanted in deep stroma developed opacities in the DM-KPro optic interface while it rarely occurred with a sDSC placed directly on DM. In burned eyes, 5 animals needed conjunctival flap repair. No eyes had epithelium downgrowth. The Eye Bank eye experiment confirmed implantation to be feasible even in cases of microperforation (3 eyes) but not with macroperforation (1 eye). Histological evaluation displayed a very thin layer of stroma (~2um) remaining on DM in 5 eyes and a minimally thin layer (~10um) in the others. Of the 9 Keralia, 8 implanted in patients having had multiple PKs, other KPros or previous extensive surgery extruded between 3 months and 1 year while one Keralia implanted in a case of decompensation not amenable to PK remains in place at ~2 years.

Conclusion The supra-Descemet Corneal Synthesis made of HEMA-MMAA34 showed promising results. As opening of the anterior chamber is not required, the sDSC theoretically offers less risks and complications than penetrating KPros. Support: European project BMH4-CT97-5907, Florida Lions Eye Bank, Research to Prevent Blindness, FWF, Austrian Science Fund, NIH Center grant P30-EY014881, and the Heron and Floris Lesieur Foundation

Commercial interest
An update on the synthetic collagenous artificial cornea

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During embryonic development, cells secrete an extracellular matrix (ECM) around themselves that is turn provides cues to the cells to direct organ development. The same ECM components would therefore also be likely to direct repair and regeneration of these organs. Our goal, therefore, was to develop biomimetic materials that are ECM analogs, capable of sustaining a native-like ECM environment and interacting with host tissues and stem cells to effect regeneration. Specifically for the cornea, we have successfully completed a 12 month study on corneal substitutes fabricated from recombinant human collagen that were implanted into mini-pigs be lamellar keratoplasty. Implants made from type I and type III recombinant collagens were compared. Both collagens could be crosslinked and moulded into the shape and size of human corneas. While we found that type III implants had a tendency to be mechanically stronger, both types of implants gave very similar results. They retained optical clarity by both slit lamp biomicroscopy and fundus imaging. They also retained their thickness indicating graft stability. Regeneration of corneal epithelial and stromal cells were also observed. In addition, corneal nerves and the tear film were regenerated. These results were comparable to those we had previously reported in shorter term studies. These recombinant collagens, as thin sheets, were also found to be suitable as scaffolds for proliferation of human corneal limbal cells. As the collagen source is recombinant, made from yeast, we have therefore shown that completely man-made corneal substitutes can be produced as substitutes of the corneal matrix. Biomimetic materials, therefore, are likely to be successful for reproducing the natural scaffolding in tissue engineering applications, such as the development of corneal substitutes.

Infection control in KPros

NOIRI M
Boston

Purpose To study Boston KPro-related infections.

Methods Retrospective review of case notes.

Results Since 1990, 350 patients have undergone Boston Keratoprosthesis surgery. An analysis has been made of those affected by bacterial or fungal infections. The results will be presented.

Conclusion Strategies to prevent and treat Boston KPro-related ocular infections will be presented.
**4321**

**Retinal pigment epithelial cells under oxidative stress**

**WEIJE-LUISSEN LI**

University Augenklinik, Munich

**Purpose** Various studies have demonstrated that cellular senescence may be accelerated by stress factors such as oxidative stress and transforming growth factor beta (TGF-β). This phenomenon has been termed stress-induced premature senescence (SIPS). The goal of the present study was to determine whether oxidative stress and TGF-β might induce SIPS in cultured human retinal pigment epithelial (RPE) cells.

**Methods** Cultured human RPE cells were exposed to hydrogen peroxide (H2O2). Additionally, cells were treated with 1 ng/ml TGF-β1 and -β2 for 48 hours. Senescence-associated beta-galactosidase (SA-β-Gal) activity was detected by histochemical staining. Expression of SA genes cyclin-dependent kinase (CDK), fibronectin, SM22 were examined by real-time PCR. Senescence-associated signal transduction proteins (p16, p21, pRB) were analyzed by western blotting. Levels of TGF-β1 and -β2 were analyzed by real-time PCR and ELISA.

**Results** H2O2 increased SA-β-Gal activity by up to 10 fold and the expression of SA genes by 3-4 folds. Treatment with TGF-β1 and -β2 showed similar fold changes. H2O2 and TGF-β1 and -β2 markedly enhanced the expression of p16 and p21. Parallel there was an downregulation of pRB expression. Neutralizing antibodies against the oxidative stress-mediated elevation of SA biomarkers.

**Conclusion** Oxidative stress and TGF-β are capable to induce SIPS in cultured human RPE cells. Therefore, reduction of oxidative stress and minimizing TGF-β may help to prevent SA changes in the RPE of early AMD.

**4322**

**Cellular responses to lysosomal dysfunction in RPE cells**

**KONTZ J**

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**Purpose** Several lines of evidence suggest that dysfunction of the retinal pigment epithelium (RPE) is a crucial event in triggering molecular pathways contributing to clinically relevant ARMID changes. Such dysfunction may be induced by phagocytosis of photoreceptor-derived compounds causing lysosomotropic effects. We therefore analysed cellular responses of cultured RPE cells to various lysosomotropic compounds.

**Methods** Cultured RPE cells were treated with 9-hydroxyxenonal, malondialdehyde, A2E, or kueptin. Their action on intralysosomal degradative capacities and intracellular storage was monitored and the resulting effects on secretion of undegraded material, autophagic sequestration and cytochrome production were analysed.

**Results** When the cells were challenged by feeding with photoreceptor outer segments, all tested compounds severely impaired lysosomal degradative functions resulting in intracellular accumulation of undegraded macromolecules and formation of lipofuscin-like material. In polarised RPE cells lysosomal dysfunction caused secretion of undegraded phagocytos-derived material at the basolateral side of the cell layer. Intracellular autophagic sequestration rates were strikingly reduced. Finally longterm lysosomal dysfunction caused increased production of stress-related cytokines, including VEG-F-A.

**Conclusion** Basolateral secretion of undegraded material observed in polarized cells suggests that an analogous pathway mechanism may also in vivo contribute to deposition of undegraded material below the RPE layer. Autophagic sequestration is considered a cellular anti-aging mechanism, therefore impaired autophagy may add to RPE aging and degeneration. Altered cytokine production induced by lysosomal dysfunction of the RPE should be further evaluated as a pathogenic factor in ARMID.

**4323**

**Proteolysis in RPE cells**

**KAARNIBANTA K**

Department of Ophthalmology, Kaupio

**Purpose** Most cellular proteins are continuously synthesized and degraded within the life-span of a cell. Two major systems take care of protein degradation in eukaryotic cells: the proteasomes, which degrade the vast majority of long- and short-lived normal and abnormal intracellular proteins and the lysosomes, which degrade membrane and endocytosed proteins. Macrophagocytosis, macrophagocytosis or chaperone-mediated autophagy are the principal forms of lysosomal degradation in mammalian cells. In macrophagocytosis, cytoplasmic structures are degraded within an autolysosomes. Connection of stress inducible Hsp70, lysosomes and proteasomes were evaluated in the protein aggregation in human RPE cells.

**Methods** Western blotting, immunofluorescence, transmission electron microscopy, confocal microscopy, hsp70 mRNA interference, GFP-Hsp70 overexpression techniques were used to study proteolysis in ARPE-19 cells.

**Results** MG-132 proteasome inhibitor caused robust accumulation of Hsp70 protein and Ub protein conjugates in ARPE-19 cells. At both the Hsp70 and Ub colocalized with the protein aggregates. The granulation of justinuclear aggregation was clearly increased in response to proteasome inhibition and simultaneous hsp70 mRNA interference. Interestingly, we found that justinuclear protein aggregation is an interim, because a cease of proteasome inhibition leads to autophagosome-mediated clearing of cytoplasmic protein aggregates. The Hsp70 colocalization was observed in both primary lysosomal fractions and in autolysosomes.

**Conclusion** The Hsp70 molecular chaperone, lysosomes and proteasomes are connected each other in macroautophagy - mediated proteolysis in human RPE cells.

**4324**

**Regulation of complement activation in ocular and vascular inflammation**

**SEPPO M**

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The complement system keeps the body clear from invading microbes and accumulating debris. Its main regulator, factor H (FH), protects host tissues and limits excessive activation. Polymorphisms or mutations in FH and other complement proteins (C3, C1r, C1s, C3a, C4b, C5b, C6) predispose to age-related macular degeneration (AMD) and other diseases with complement-mediated tissue damage: membranoproliferative glomerulonephritis type II (MPGN II) and recurrent atypical hereditary urticarial syndrome (aHUS). AMD-associated polymorphism in the middle part of FH influences the ability of FH to bind to C-reactive protein (CRP). This would affect phagocytic clearance of debris (e.g. eye pigment) and cause local inflammation. Accumulation of pigment, protein aggregates and stress conditions in retinal pigment epithelial cells provide a challenge to complement-mediated clearance. Excess cellular stress or regulatory complement disturbances sensitize tissues to complement attack and inflammation. An interplay between CRP and complement is seen also in atherothrombotic vasculitis. Loss of the ability of FH to recognize C1b molecules deposited of polyanion-rich self structures predisposes to aHUS, where blood cells, platelets and endothelial cells, particularly in kidneys, become targets for repeated complement attacks. The pathogenetic mechanisms of these diseases are being increasingly understood, which will help in designing new therapies.
### 4332
**Misinterpretation of common ophthalmic tests**

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**Neuro-Ophthalmology, Lausanne**

**Purpose** In order to solve the case of a patient with a neuro-ophthalmic disorder, paraclinical tests are frequently needed. They include evaluation of the visual fields, electroretinography, visual evoked cortical potentials to name a few. Without a careful examination of the results, the clinician might be misled in the diagnosis and the care of the patient might be inappropriate.

**Methods** Illustrative cases will be presented in an interactive way.

**Conclusion** At the end of the session, the participant will be aware of some of the “traps” that can be avoided with a thorough examination of such test results.

### 4333
**Radiologic pitfalls in neuro-ophthalmology**

**Milea D**

GlASTER UNiveRsity Hospital, Copenhagen

**Purpose** Although standard neuro-imaging methods have a major contribution to appropriate diagnosis in neuro-ophthalmology, they do not represent “one size fits all” procedures. Structural lesions can sometimes escape detection, mainly because of suboptimal clinical information for the radiologists.

**Methods** This presentation will illustrate some common errors in prescription or interpretation of neuro-imaging in neuro-ophthalmology.

**Results** Several protocols are used by each center depending on the explored pathology (multiple sclerosis, aneurysms, carotid dissection) or the anatomical location of the condition (orbit, cavernous sinus, sella, etc.). When an MRI is indicated, application of intravenous contrast is not routine; especially if the expected lesion is supposed to be large enough. Contrast injection may be mandatory for detection of small lesions, even though this increases study acquisition time and is more expensive. Fat suppression is critical for detection of small lesions located in areas containing fat, such as orbits, or neck. Multiple, thin, coronal images are very useful for visualization of the chiasm, cavernous sinus and the possible surrounding compressive structures (i.e. pituitary adenoma, small meningioma). Specially dedicated sequences explore the white matter in multiple sclerosis or can find abnormalities in early ischemic events, while standard MRI can overlook these lesions. One can not perform all these protocols in every patient, the choice being dependent on the clinical information, the technical availability and the local expertise.

**Conclusion** Appropriate neuro-imaging can result only from clear, informative clinical indications about symptoms with localizing value, whenever possible.

### 4334
**Management misadventures**

**Boschi A**

**Ophthalmology, Brussels**

**Purpose** Neuro-ophthalmic diseases have the potential for delay in diagnosis, misdiagnosis, or mismanagement.

**Methods** The presented clinical cases will cover optic neuropathy such as arteritic and non-arteritic ischemic optic neuropathy, idiopathic intracranial hypertension, Graves’ disease, sarcoidosis, myasthenia and others. Through the clinical cases of the common neuro-ophthalmic disorders, we will discuss the common mis-steps in the clinical and imaging diagnostic approach. We will also consider the consequences of delay diagnosis, and the importance of an early treatment will be highlighted. For each case, we will review the clinical diagnostic criteria and the management recommendations will be discussed.
**4341**

The basics of wavefront aberrometry

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Wavefront technology has been first used by astronomers for years in order to reduce higher order aberrations induced by the earth's atmosphere; it has been introduced into clinical eye care recently. Ophthalmological practice involves performing a full subjective refraction. The sphere, cylinder and axis of astigmatism are measured. We are only correcting two components of a whole host of refractive components of the optics of an eye. These two components (sphere and cylinder) constitute by far the majority of the optical aberration of an eye. Even a basic exam yields important information about optical quality. However, all ophthalmologists have been faced with patients reporting visual acuity (or contrast sensitivity, glare, etc.) at levels much lower than would be expected from an eye exam. Wavefront aberrometry measures aberrations over the entire eye taking into account not only spherocylindrical refractive error, but also spherical aberration, trefoil, coma, secondary astigmatism as well as other 'higher order' aberrations described by Zernike polynomials. Higher order aberrations are thought to contribute to more than 20% of the total number of aberrations in a normal eye an increase with pupil size. In the majority of normal patients, these high order aberrations play a minor role; however, in cases of refractive surgery, keratoconus and orthokeratology, they can induce a number of visual disturbances. We will define higher-order aberrations and show how to measure them, giving you a basic working knowledge of wavefront sensing (also known as aberrometry). We will show how wavefront sensors work, what are Zernike polynomials, what they tell us and we will show present and future clinical applications of wavefront aberrometry.

**4342**

Applications of adaptive-optics in ophthalmology

CHATEAULIN

Imagine Eyes, Orsay

Purpose This presentation reviews the current and potential applications of adaptive optics (AO) in the area of ophthalmology.

Methods AO was invented by astrophysicists in order to eliminate image blur and twinkling in large terrestrial telescopes. This technique cancels the optical distortions that are introduced by the earth's atmosphere, a turbulent and inhomogeneous medium, in the light emitted by distant stars. Like the atmosphere, the human eye is optically imperfect: it suffers from wavefront aberrations whose pattern and amount differ between individuals. This is the reason why AO has met several applications in ophthalmology. One part of the AO technology consists in measuring the distortions of light using a wavefront sensor: this technique has been integrated into wavefront aberrometers that routinely improve the precision of LASIK procedures.

Results The full implementation of AO has recently led to other breakthrough devices. AO visual simulators are enhanced aberrometers that allow patients to visualize, at the pre-operative stage, the expected outcome of refractive surgery procedures, including preoperative ablations. Several research laboratories have implemented AO to improve the resolution of various retinal imaging techniques (fundus camera, SLO, OCT) up to the scale of single retinal cells. Three-dimensional images of cone photoreceptors have recently been obtained with a resolution of 3µm, both in the longitudinal and transverse dimensions of the retinal tissue. Aside from this world-record, the presentation will also address the limitations of the technique and describe some of the work in progress towards the clinical applications of AO technology.

Conclusion AO technology will likely provide new devices that meet unsatisfied needs in refractive surgery and retinal diagnostics.

Commercial interest

**4343**

Sports and wavefront aberrometry

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Purpose In this paper we study the future applications of wavefront aberrometry in high-level sportsmen and in Tennis pros in particular.

Methods We compare the level of ocular aberrations between a group of international junior french tennis players from the « Pôle France » to Amateur Tennis Players of the same age, with a Shack Hartman aberrometer: IRX 3 (Imagine Eyes ). We try to find if in the tennis pros group there is a correlation between the level of ocular aberrations and athletic performance.

Results Compared to the literature data on normal subjects, the global level of ocular aberrations (total RMS) of professional tennis players is very low, but is not statistically different compared with our control group (amateur tennis players). Though non corrected monocular and binocular vision acuity is higher in the tennis pros group, they total RMS is not statistically different from the one of amateurs. However professional tennis players present with less high order aberration than amateurs. We found a significant correlation between the level of ocular aberrations and tennis performance in the International tennis players group.

Conclusion We found that the specific population of young international tennis players seems to have a natural « Supervision ». The good visual performances of these subjects are not correlated with a complete absence of ocular aberrations, but they present with very low level of high order aberrations. Associations of selected ocular aberrations might even be beneficial. Aberrations distribution in this population may help define new standards for specific sport aptitudes.

**4344**

Limitations of correcting aberrations with soft contact lenses

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Purpose In this paper, we study the limitations of correcting aberrations with soft contact lenses, which are custom-designed for each subject aberrations with special interest on the effect of measuring and correcting wavefront in different planes (pupil vs cornea plane).

Methods We analyze, by experiments, theoretical methods and computer simulations, issues such as aberration dynamics, lens movement and errors in pupil plane localization. The study is carried out for three types of eyes: normal, keratoconus, and eyes that have gone through penetrating keratoplasty.

Results Main factor limiting this type of aberration correction is the static horizontal and vertical translation as well as the rotation of the contact lenses on the eye. Typical rotations (3°, 5°) and translations (0,5 mm) of the soft contact lenses could null the benefit of the high-order corrections in normal eyes. The correction of the wavefront in a different plane were it has been measured is not negligible when the aberrations (low and high order) are high.

Conclusion We found that customized correction for normal eyes leads to a loss of optical quality; compared to standard correction (sphere and cylinder), when displacements of the contact lenses appear. In spite of the mentioned effects, there are, however, pathologic cases in which it is possible to reduce the RMS and increase visual quality, especially in the keratoconus eyes. In those high aberrated eyes special care should be taken due to the changes of the wavefront when propagating from the cornea plane to the pupil plane.
Wavefront guided custom ablation: facts and fiction

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Wavefront guided customized ablation is an exciting new tool for the modern refractive practice. Wavefront aberrometry gives us an accurate "fingerprint" of the eye. However, we should ask ourselves the following questions: First, can we improve visual acuity outcomes? Secondly, how can we or can we avoid and fix high order aberrations? In this presentation we describe in detail the new optical challenges of refractive surgery. Through a review of the literature and based on personal experience we show you the basics of custom wavefront technology, the promises but also the potential pitfalls of aberration guided laser ablation.
**SIS: Matrix metalloproteinases in vascular and ocular disease**

- **4351**
  MMPs in vascular disease
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  **ABSTRACT NOT PROVIDED**

- **4352**
  MMPs in ocular physiology
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  **Purpose** Matrix metalloproteinases (MMPs) are a family of more than 20 enzymes, with the common ability to degrade extracellular matrix (ECM) components. Since MMPs may also destruct signaling molecules or release them from ECM, MMPs shape not only the structural, but also the functional aspects of a distinct ECM compartment. As a consequence MMPs are implicated in a wide range of physiological and pathological processes in the eye (e.g. corneal wound healing, AMD, diabetic retinopathy, choroidal neovascularization, and glaucomatous changes in trabecular meshwork and optic nerve head). This review will summarize our knowledge of MMPs in ocular tissues and our present understanding of the regulation of MMP activity.

  **Methods** Review of the literature.

  **Results** MMPs are zinc endopeptidases which can be subdivided in 4 subgroups. Collagenases process fibrillar collagen types I, II and III. Gelatinases degrade basement membrane collagen types IV and V. Stromelysins recognize nonfibrillar collagens, laminin, fibronectin and proteoglycans. Recently, membrane type MT-MMPs were detected bound to cell membranes. MMP activity is controlled basically on three levels. Synthesis is regulated by transcription factors. Secreted, inactive proMMPs must be activated, mainly by plasmins. Plasmin itself is regulated by plasminogen activators (PA) and their counterpart plasminogen activator inhibitors (PAI). Finally, active MMPs may be blocked by tissue inhibitors of MMPs (TIMPs).

  **Conclusion** The variety of MMPs, their ubiquitous presence and their broad range of substrates develop a complex system with tight regulation on several levels. To date our understanding is far from satisfying and further work is necessary to reach a more comprehensive look especially at the biological impact of this family of enzymes.

- **4353**
  MMPs in CNV
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  **Purpose** Matrix metalloproteinase 2 (MMP-2) and MMP-9 are increased in human choroidal neovascularization (CNV) occurring during the exudative most aggressive form of age-related macular degeneration (AMD), but their precise role and potential interactions remain unclear.

  **Methods** To address the question of MMP-2 and MMP-9 functions, mice deficient in the expression of MMP-2 (MMP-2 KO), MMP-9 (MMP-9 KO), and both MMP-2 and MMP-9 (MMP-2,9 KO) with their corresponding wild-type mice (WT) underwent CNV induction by laser-induced rupture of the Bruch's membrane.

  **Results** Both the incidence and the severity of CNV were strongly attenuated in double deficient compared with single gene deficient mice or corresponding WT controls. The reduced neovascularization was accompanied by fibroin/fibrin accumulation. Furthermore, overexpression of the endogenous MMP inhibitors TIMP-1 or TIMP-2 (delivered by adenoviral vectors) in WT mice or daily injection of a synthetic and gelatinase specific MMP inhibitor (Ro 26-2835) significantly decreased the pathological reaction.

  **Conclusion** These findings suggest that MMP-2 and MMP-9 may cooperate in the development of AMD and that their selective inhibition represents an alternative strategy for the treatment of choroidal neovascularization.

- **4354**
  MMPs in glaucoma
  SCHLOETZER SCHREHARDT U
  Department of Ophthalmology, Erlangen

  **Purpose** Extracellular matrix turnover is mediated by matrix metalloproteinases (MMPs), a large family of endopeptidases with variable substrate spectra. The activity of these enzymes is regulated in part by specific endogenous inhibitors, the tissue inhibitors of metalloproteinases (TIMPs). Dysregulated expression of MMPs and TIMPs has been implicated in many ocular disease processes accompanied by abnormal matrix production including glaucoma. The purpose of this review is to highlight recent insights into the roles of MMPs and TIMPs in the regulation of aqueous humor outflow and retinal ganglion cell death in chronic open-angle glaucoma.

  **Methods** Basic research and review of the literature.

  **Results** MMPs have been shown to affect matrix remodeling and outflow resistance in the trabecular meshwork, and to influence ganglion cell fate by matrix remodelling and glutamate receptor processing in the retina. Several current glaucoma therapies including topical prostaglandin analogues and laser trabeculoplasty have been shown to induce MMP activation in the outflow pathways. Changes in the expression of MMPs and TIMPs have been also shown to be involved in conjunctival wound healing after glaucoma filtration surgery.

  **Conclusion** Modulation of MMP activity may provide an attractive adjunctive treatment option to improve outflow facility, to attenuate retinal ganglion cell loss, and to reduce conjunctival scarring in glaucoma patients.
*4361
Choroidal tumor biopsy: a prospective case series
KHIRSHID G, COUPLAND S, DAMATO B
Liverpool
ABSTRACT NOT PROVIDED

*4362
Endoresection of choroidal melanoma after proton beam radiotherapy
KHIRSHID G, GROENWALD C, DAMATO B
Liverpool
ABSTRACT NOT PROVIDED

*4363
In vivo prognostication of posterior uveal melanoma: cost effectiveness and clinical value of different molecular tests
PARROZZANI R, MIDENA E
Pavia
ABSTRACT NOT PROVIDED

*4364
An European protocol for the detection and treatment of high risk uveal melanoma
DESIARDINS L, COUTURIER J, PIPERNO-NEUMANN S
Paris
ABSTRACT NOT PROVIDED
4365
Correlation between ultrasonographic tumor data and peritumoral subretinal fluid findings with OCT for the early detection of small malignant melanocytic tumors

GRANGE J-D, GARNIER S, JEAN-LOUIS R, ABF-AYAD N, KODIKI AN L

ABSTRACT NOT PROVIDED

4366
Validating the Helsinki University Central Hospital (HUH) working formulation for staging metastatic uveal melanoma

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7. St Paul's Eye Unit, Royal Liverpool University Hospital, Liverpool
8. Centre Pluridisciplinaire d'Oncologie and Hospital Jules Gonin, University Hospital, Lausanne
9. for the European Ophthalmic Oncology Group

Purpose
To validate the Helsinki University Central Hospital (HUH) Working Formulation, the first substaging system designed to predict prognosis of metastatic uveal melanoma and to facilitate design, reporting and interpretation of controlled clinical trials.

Methods
Data of 226 patients who died of disseminated uveal melanoma were collected from members of the European Ophthalmic Oncology Group (OOG). Karnofsky or WHO performance status, largest diameter of largest metastasis and serum alkaline phosphatase (AP) level at diagnosis of metastases, and time to death were requested. The working formulation is based on a multivariate model that identified these variables as independent predictors of survival, modeling time on chemotherapy as a confounding variable (Eskelin et al. Cancer 2003; 97: 65–75). Patients whose predicted survival was less than 6 months were assigned to stage IVc, between 6 and 12 months to stage IVb and over 12 months to stage IVa. Observed survival time was calculated for each stage.

Results
The median AP level was 0.69 (range: 0.2–11) times upper normal limit, median largest metastasis was 3.5 cm (range: 0.8–19), and percentage of patients representing WHO performance index 0, 1–2 and 3 was 61%, 38% and 1%, respectively. Of the 226 patients, 100 (44%) were assigned to stage IVa, 97 (43%) to IVb and 29 (13%) to IVc. The median observed survival times were 18.3, 10.0 and 4.6 months, respectively. In Kaplan–Meier analysis, the stages reflected best to worst survival (P=0.0001, log-rank test for trend).

Conclusion
The collaborative OOG study confirms the HUH Working Formulation as a repeatable, valid system for dividing patients with metastatic uveal melanoma into groups of differing prognosis.
**4411**

**Corneal Cross Linking**

SEILER T
IROC, Zurich

**ABSTRACT NOT PROVIDED**

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**4412**

**Aspects of safety of riboflavin-UVA crosslinking of the cornea**

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(2) IROC, Zurich

**Purpose** To investigate potential damages to ocular tissue during corneal collagen crosslinking (X-linking) by means of the riboflavin/UVA (370 nm) approach.

**Methods** Comparison of the currently used technique with officially accepted guidelines regarding direct UV-damages (without riboflavin) and the damages created by the induced free radicals (photochemical damage).

**Results** The currently used UVA dose density of 5.4 J/cm² and the corresponding irradiance of 3 mW/cm² without riboflavin is below the known damage thresholds of UVA for the corneal endothelium, lens, and retina. Regarding the photochemical damages due to the free radicals, the damage thresholds for keratocytes and endothelial cells are 0.45 mW/cm² and 0.35 mW/cm². In a 400 µm thick cornea saturated with riboflavin the irradiance at the endothelial level is 0.18 mW/cm² which is a factor of 2 smaller than the damage threshold.

**Conclusion** After corneal X-linking the stroma is depopulated of keratocytes approximately 300 microns deep. Repopulation of this area takes up to 6 months. As long as the cornea treated has a minimum thickness of 800 microns (as recommended) the corneal endothelium will not experience damages nor will deeper structures such as lens or retina. The light source should provide a homogenous irradiance avoiding hot spots which may locally destruct the endothelium.

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**4413**

**Corneal molding and crosslinking**

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Banco Institute, Barcelona

**Purpose** To develop an experimental setup in human donor eyes to study the feasibility of combining corneal crosslinking (CCL) with molding techniques such as orthokeratology and intracorneal arcuate implants.

**Methods** 6 pairs of fresh human donor eyes with corneas unsuitable for transplantation but otherwise normal were maintained at constant 20 mm Hg intraocular pressure (IOP). The epithelium was removed and an orthokeratologic PMMA contact lens of 11.8 mm diameter and 10 mm main curvature radius was fixed to one cornea of each pair with 6 sutures of 9.0 vicryl. A standard CCL protocol (0.1 riboflavin phosphate, 370 nm UVA at 3 mW/cm² for 30 minutes) was applied simultaneously to both corneas. We measured pre- and postoperative IOP, central pachymetry, corneal topography (EyeSys), corneal hysteresis (CH) and resistance factor (CRF) (ORA).

**Results** Average topographic cylinder (SimK) went from 1.66 diopters (D) to 2.01 D and from 1.55 to 1.06 D pre- to postoperatively, respectively in the orthok-CCL and CCL-only corneas. Average maximal K value changed from 44.8 to 43.7 D in the orthok-CCL and from 43.8 to 43.4 in the CCL-only group. None of these differences was statistically significant. Average CH went from 11.0 to 13.4 and from 9.0 to 13.7, respectively in the orthok-CCL and CCL-only groups, while CRF went from 11.9 to 14.9 and from 10.7 to 14.6 respectively. Both groups showed a significant increase in pre- to postoperative CH and CRF, without significant differences between them.

**Conclusion** An experimental setup has been devised, able for testing combined CCL and corneal molding techniques in donor human eyes. Corneal molding by a sutured standard orthokeratology contact lens combined with CCL does not appear to induce a permanent change in corneal curvature parameters.

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**4414**

**Riboflavin/Ultraviolet a corneal collagen Cross-Linking: one-year results**

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**Purpose** To evaluate one-year results of riboflavin/ultraviolet A corneal collagen Cross-Linking (CL) in 28 eyes with progressive keratoconus.

**Methods** Refraction, visual acuity, pachymetry, corneal topography and wavefront analysis were evaluated preoperatively and at one year after CL.

**Results** At year one, topographic and subjective astigmatism decreased, respectively from 4.70 to 4.44 D and from 3.03 to 2.91 D. Wavefront analysis showed coma reduction from 1.90 to 0.76µm. UCVA increased from 0.15 to 0.25 and BSCVA from 0.45 to 0.06. Cone area, cone diameter, apical keratometry and curvature gradient remained stable.

**Conclusion** Collagen CL appears to be a safe procedure to stabilize keratoconus and to reduce aberrometric comatic component.
4415
Ocular response analyzer findings in keratoconic eyes undergoing Cross Linking procedure
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Purpose To evaluate the effect of corneal collagen Cross Linking on IOP, corneal hysteresis (CH) and corneal resistance factor (CRF) using the Ocular Response Analyzer.

Methods Twenty keratoconic eyes underwent cross linking procedure after epithelium removal. Eyes were studied by means of Ocular Response Analyzer before cross linking, after epithelium removal, after cross linking and at 3 months follow up. IOPs, IOPg, CH and CRF were analyzed.

Results At 3 months follow up mean IOPs was found to be 15.78 mmHg, significantly higher than the mean IOPg, which was found to be 11.33 mmHg. Corneal Resistance Factor and Corneal Hysteresis were found to be significantly low after epithelium removal and higher than the preoperative value after cross Linking procedure.

Conclusion The device allows us to study corneal biomechanical properties before and after cross linking and to follow over time the effect of corneal photodynamic therapy in strengthening the stromal collagen fibrillae.

4416
Crosslinking in iatrogenic keratectasia after LASIK: pregnancy-related exacerbation
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Purpose Corneal collagen crosslinking can arrest the progression of LASIK-induced keratectasia. We report a patient who developed bilateral iatrogenic keratectasia only during her first pregnancy at three years after initial LASIK. We performed bilateral corneal collagen crosslinking. In the following two consecutive years, keratectasia remained stable and even regressed. Upon her second pregnancy at two years after crosslinking, keratectasia exacerbated again.

Methods We performed corneal collagen crosslinking with riboflavin and UVA in March 2005 (right eye) and April 2005 (left eye) as described previously.

Results UV-induced crosslinking led to an arrest and regression of keratectasia progression over a postoperative follow-up period of 22 months, which was demonstrated by stable pre- and postoperative corneal topography and reduction of maximal K readings. However, in the fifth month of the patient's second pregnancy, keratectasia exacerbated again.

Conclusion We report the first case of exacerbation of keratectasia after corneal collagen crosslinking. Remarkably, exacerbation occurred during pregnancy indicating that hormonal changes might affect biomechanical stability of the cornea.

4417
Micromorphological analysis of cross-linked cornea by in vivo scanning laser
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Purpose To assess in vivo micromorphological corneal modifications and healing process after Riboflavin-UVA collagen cross-linking in 44 human patients with progressive keratoconus, by means of HRT II scanning laser system confocal microscopy.

Methods Second phase prospective non randomised open study in 44 keratoconus patients treated and assessed as described in PURPOSE at Siena University. Treatment in topical anaesthesia included a 9 mm diameter epithelial debridement, instillation of 0.1% Riboflavin phosphate - 20% dextran T 500 solution 15 minutes before UVA irradiation and every 5 min. Radiant energy was 3 mW/cm2 or 54 joule/cm2 min, exposure time 30 min. The source was a led UVA illuminator 370 nm developed by Caporossi Barocchi and Mazzaotta X Linker (CSO). After removal of the soft contact lens, all patients were assessed by HRT II system confocal microscopy in vivo at 1, 3, 6 and 12 months.

Results Disappearance of stromal nerves was observed in the central irradiated area of 9 mm. Six months after the stroma was recolonised by nerve fibres with restoration of normal corneal sensitivity clinically detectable. Rarefaction of keratocytes in the anterior-mid stroma, associated with edema was observed. Keratocytes repopulation in the central treated start 3 months after the operation becoming complete at about 6 months. The increased reflectivity of extracellular matrix in all cases represent the most important indirect sign of a crosslinked cornea.

Conclusion HRT II system confocal microscopy confirms in vivo corneal reinnervation, keratocytes repopulation of the stroma and the real depth of treatment. Healing process, limbal stem cells and transient haze were also analyzed for a complete safety evaluation of the method.

4418
Mid term results in keratoconus treatment by Riboflavin – UVA corneal collagen Cross-Linking. Siena eye cross project
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Purpose We present a mid term follow up analysis of 44 keratoconic eyes treated by combined Riboflavin-UVA collagen cross-linking.

Methods Progressive keratoconic eyes of 44 patient were treated by combined Riboflavin-UVA collagen cross-linking. UVA exposure was delivered by the new CIBM X-linker 370 nm Led array CSO, Italy. Riboflavin 0.1% solution, Ricrolin Soft, after corneal epithelium debridement in all cases was instilled 15 minutes before starting irradiation and every 5 minutes for 30 minutes. Eyes were dressed with a soft contact lens for 4 days and medicated with levofloxacin drops, flurbiprofen drops and lacticram substitutes for 15 days.

Results In all treated eyes no progression of keratoconus was detected, the control fellow eye progressed in 30 % of cases in one year follow up. Mean K value improved meanly 2 Diopters with a significant reduction of Coricam aberration, improving corneal simmetry and quality of vision.

Conclusion Corneal crosslinking is indicated in progressive keratoconus particularly in young patients under 26 years. It can delay or stop keratoconus worsening reducing necessity of donor corneal transplantations.
Diabetic retinopathy screening with nonmydriatic fundus camera (1 and 3 fields) vs ETDRS standard

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Purpose To evaluate the ability to determine single lesions and to grade clinical levels of diabetic retinopathy (DR) using nonmydriatic (NM) digital color retinal images compared to Early Treatment Diabetic Retinopathy Study (ETDRS)/7 standard 35-mm stereoscopic color fundus photos.

Methods 80 eyes of 42 diabetic patients with wide spectrum of DR lesions were enrolled. Non-simultaneous 45°-field non-stereoscopic digital color images were taken from 1 central field (1F) and from 3 fields (3F) with a NM automatic fundus camera (Nidek Technologies, Italy). After dilatation standard 30° ETDRS photos were obtained with Topcon TRC-50AI (Japan). The images obtained were analysed by 2 independent, masked readers evaluating lesion by lesion to assess the ETDRS grading and the clinical severity of the disease according the International proposed 5-stage disease severity classification.

Results Comparison of single lesions among 1F or 3F images and ETDRS fields revealed good agreement for hard exudates (k=0.74 and 0.75 respectively), moderate agreement for hemorrhages and/or microaneurysms (k=0.49, k=0.60), cotton wool spots (k=0.41, k=0.49), and retinal new vessels (k=0.40, k=0.43). The agreement was fair for clinically significant macular edema (k=0.40, k=0.39). There was substantial agreement between 3F and ETDRS images for moderate non-proliferative and proliferative DR (k=0.70 and k=0.89 respectively), while it was moderate between 1F and ETDRS images.

Conclusion The automatic non-mydriatic 3 field system may be an effective tool to determine the level of DR and to identify the need for prompt referral to the ophthalmologist, even when applied as a telemedicine tool. It is still controversial if one 45° image is adequate for DR screening.

Diabetic macular edema

Free Papers: Diabetic macular edema

Diabetic related complications – the Moorfields Diabetes Survey

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Purpose The aim of this study was to establish characteristics of patients with diabetes mellitus (DM) attending Moorfields Eye Hospital (MEH) before implementation of the national diabetic retinopathy (DR) screening programme.

Methods Patients with DM attending MEH Medical Retina Clinics during a 4 week period were interviewed about DM, its complications and knowledge and expectations on laser treatment.

Results In 4 weeks, 830 patients with DM were identified, of whom 4.3% were registered partially sighted and 4.7% registered blind. 141 did not attend, 109 could not be interviewed and 580 interviews were conducted. Interviewed patients were similar to those not except for previous laser treatment (p=0.016). The median duration of DM was 10 years (0.3-52 years); 74.3% had Type 2 DM, 64.8% had hypertension. A high proportion of patients had at least one diabetic complication (heart disease 24%, stroke 9.7%, diabetic neuropathy 48.9%, diabetic nephropathy 13.4%); 13.1% did not understand the relationship of DM with DR. In the past 61.5% and 64.1% patients had fluorescent angiography (FFA) and laser treatment, on the day of the interview 3.8% had FFA and 13.4% laser. Despite full consent, 51.6% did not understand how long it took the laser treatment to work or expected it to take effect immediately, 16.6% expected to have only one treatment ever. Only 20.9% were members of Diabetes UK.

Conclusion Patients currently attending eye clinics have many diabetes related complications. With full implementation of the National Screening Programme for DR this percentage is expected to rise. Careful planning of ophthalmic services needs to be undertaken to accommodate the need for services for patients with multiple diabetes related complications.

Retinal fixation in Diabetic Macular Edema

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Purpose Diabetic macular edema (DME) determines central retinal sensitivity reduction with a functional correlate to increased macular thickness. Data about fixation are controversial. The aim of this study was to determine fixation characteristics in diabetic patients with or without clinically significant macular edema (CSME).

Methods 120 consecutive diabetic patients (165 eyes) underwent best corrected visual acuity (BCVA) determination (ETDRS charts), and a complete ophthalmologic examination. Diagnosis and classification (focal, diffuse and cystoid) of CSME were made on 30 degrees stereo retinal photographs (field 2 of ETDRS) and fluorescent angiography and OCT. The presence and site of hard exudates were evaluated. Retinal fixation and macular sensitivity were investigated with an advanced, automatic micropimeter (MFP, Nidek Technologies, Italy). Macular thickness and foveal neuroretinal detachment were determined by Optical Coherence Tomography (OCT Stratus, Zeiss Humphrey Instruments, Germany).

Results Of 141 examined eyes 24 (17.14%) had focal CSME, 37 (26.43%) had diffuse CSME, 51 (38.43%) cystoid CSME, 29 (19.76%). Mean BCVA was 0.06-0.36 logMAR. There was no significant correlation between the type of edema and the site and the stability of fixation (P=0.05). Fixation was eccentric or unstable in case of subfoveal hard exudates (Site P=0.032, Stability P=0.0113). The presence subfoveal neuroretinal detachment did not significantly influence fixation pattern (P=0.05).

Conclusion Retinal fixation patterns in DME vary differently based on clinical characteristics of foveal involvement. Fixation may be often located in areas undergoing laser treatment. The functional impact of focal photocoagulation should be investigated also from the fixation point of view.

Decreased OCT measured central retinal thickness in patients with and without minimal diabetic retinopathy

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Purpose To evaluate OCT measured central retinal thickness (RT) in diabetes mellitus (DM) patients, with or without minimal diabetic retinopathy (DR).

Methods DM patients with or without minimal DR on biomicroscopy, were included and underwent a full ophthalmologic examination, (redfree) fundus photography and OCT scanning of the macula using StratusOCT (Zeiss). Mean RT measurements of the fovea (A1), pericentral ring (A2-A5) and the peripheral ring (A6-A9) in the patients were compared with RT measurements in normal, sex and age-matched subjects.

Results One hundred DM patients were included in this study of which 50 type 1 and 50 type 2. Fundus photography showed either no abnormalities or only few microaneurysms in the posterior pole. The mean pericentral RT was significantly decreased in DM patients with and without minimal DR compared with the normal controls (n=100). The predicted value of the mean pericentral RT calculated with a regression model, based on the normal subjects, was not significantly different from the truly measured mean pericentral RT.

Conclusion In contrast with previous studies, we found that the pericentral RT in DM patients seems to be decreased compared to healthy subjects. This could be explained by the loss of intraretinal neurons in the earliest stage of DR. The use of a regression model suggests this neural tissue loss is not restricted to the pericentral region.
Diff erence in laser treatment decisions for diabetic macular edema, based on biomicroscopy or OCT

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Purpose Comparison between laser treatment decisions for diabetic clinical significant macular edema (CSME) diagnosed by slitlamp biomicroscopy or OCT (Stratus)

Methods Diabetic patients (n=25) with a clinical suspicion of the presence of clinical significant macular edema (CSME) were examined by stereo slitlamp biomicroscopy and OCT for the exact location and extent of CSME. Results of these observations were drawn into a set of FA images (early / mid / late phase). Independent observers made decisions regarding laser treatment (position, pattern, and number of laserspot) based on these images.

Results The diagnosis of CSME differed between the two methods in 11 of the patients. Treatment decisions based on these different observations differed with respect to the amount and localisation of the laserspots

Conclusion A diagnosis of CSME in diabetic patients differs comparing slitlamp biomicroscopy with OCT, and treatment decisions based on either method likewise differed.

Two-year follow-up of patients with Diabetic Macular Edema (DME) treated with pegaptanib (Macugen)

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Purpose The results of a 36-week, phase 2 trial of pegaptanib (Macugen) for DME suggest pegaptanib improves visual acuity (VA), reduces retinal thickness, and causes regression of neovascularization (NV). The results of an extended follow-up after pegaptanib discontinuation are reported.

Methods This multicenter, double-masked, controlled study included subjects with best-corrected VA between 20/50 and 20/120 in the study eye and DME involving the center of the macula. Subjects were randomized (1:1:1:1) to intravitreous pegaptanib (0.3, 1, 3 mg) or sham injections at study entry and weeks 6 and 12. Additional pegaptanib injections and/or focal/grid laser photocoagulation were administered as needed for another 18 weeks; thereafter, only photocoagulation was given at the investigators discretion. Study endpoints were evaluated at weeks 36, 52, and 82.

Results At baseline, treatment groups (total N=172) were well balanced for VA and retinal thickness. At weeks 36, 52, and 82, mean VA change from baseline was better for subjects receiving 0.3 mg (n=44) versus sham (n=42) (+4.7 vs -0.4 letters, P=0.04; +3.6 vs -0.3 letters, P=0.15, +1.5 vs -2.4 letters, P=0.23). At each time point, more subjects receiving 0.3 mg gained +10 and +15 letters and had a greater reduction in mean central retinal thickness (-68 vs -37 µm, -82 vs -37 µm, -122 vs -49 µm). Retinal NV at baseline showed regression at week 36 in 8/13 (62%) eyes. Treatment with pegaptanib was well tolerated.

Conclusion The relative treatment benefit and safety of pegaptanib in DME achieved at week 36 appears to remain evident through 2 years even after injections are discontinued. Further trials of pegaptanib for DME appear to be warranted.

Commercial interest
**Correlation between saccadic parameter variations and clinical evidences in thyroid autoimmune ophthalmopathy (TAO) affected patients**

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**Purpose** Object of our study is to value correlation between saccadic parameters, estimated through EOMG exam, and several clinical symptoms/signs in subjects affected by TAO.

**Methods** 80 eyes of 40 patients consecutively affected by TAO were examined. All the patients were submitted to ocular motility exam, Hertel exophthalmometry, clinical evaluation and saccadic movement analysis through Electromyography (EOMG, IM6000MOE system - WinEOMG software ). CAS score was calculated in each subject.

**Results** Most of observed patients showed at least a grade mild exophthalmos. In 40% of observed patients we found an increase of saccadic movement maximal speed; in 30% we found a decrease of movement medium speed; 25% of observed patient showed an increased latency. Almost all subjects with any saccadic alteration showed exophthalmos.

**Conclusion** In TAO affected patients seem not to be correlation between CAS score and saccadic alterations or exophthalmos entity. On the contrary, there seem to be a correlation between saccadic alterations and exophthalmos. Even if these data still need to be confirmed, analysis with EOMG could bring an ocular motility alteration predictive value.

**Methods for quantifying diplopia**

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**Purpose** Methods for quantifying diplopia include 1) the Goldmann diplopia field, 2) the Cervical Range of Motion (CROM) method, evaluating 10 specific positions of gaze and 3) a Diplopia Questionnaire addressing 7 positions of gaze (rating diplopia as always, sometimes or never). We investigated how patients' subjective perception of the severity of their diplopia (percent impact) related to scores obtained using each of these methods.

**Methods** 28 patients with acquired binocular diplopia due to strabismus were asked to rate the percent impact of their diplopia, based on all daily activities. Diplopia was also quantified using the Goldmann perimeter, CROM method and Diplopia Questionnaire. Scores were scaled from 0 to 100, where 0 was no diplopia and 100 was diplopia in all measured positions. Correlations between percent impact and diplopia measures were assessed using intra-class correlation coefficients (ICC) and mean values compared using t-tests.

**Results** Across all patients, the mean self-rated percent impact was 40% (range 0 to 100%). Mean scores using the CROM and Questionnaire were similar to percent impact (39% and 38% respectively), whereas Goldmann scores significantly overestimated severity when compared to perceived impact (51%, p<0.05). Percent impact was most closely correlated to the Questionnaire score (ICC=0.75). The most frequent reason for a higher Goldmann diplopia score was scoring of diplopia beyond 30 degrees.

**Conclusion** Quantifying diplopia using the CROM method and Diplopia Questionnaire better represents the perceived impact of patients' diplopia than scores obtained on the Goldmann perimeter. The CROM method and Diplopia Questionnaire may prove useful instruments for clinical evaluation of dipoptic patients and in future clinical trials.

**The development of psychiatric disease in young adults who had childhood strabismus**

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**Purpose** Although adverse psychosocial effects are common in patients affected with strabismus, no study has shown an association of strabismus and mental illness. We investigated the prevalence and types of psychiatric disorders diagnosed by early adulthood among patients who had strabismus as children.

**Methods** The medical records of all children (<19 years) diagnosed with esotropia (n=266) or exotropia (n=141) as residents of Olmsted County, Minnesota from January 1, 1985, through December 31, 1994, were retrospectively reviewed for the diagnoses of psychiatric disease. Each case was compared with a randomly selected one-to-one birth- and gender-matched control from the same population.

**Results** A mental health disorder was diagnosed in 181 (44.5%) of the 407 patients with a history of childhood strabismus followed to a mean of 17.4 years compared to 135 (31.2%) of controls (P<0.001). Children with exotropia were 29% (CI 1.8-4.7) times more likely to develop a psychiatric disorder than controls when followed to a mean of 20.3 years. Esotropic children were no more likely to develop mental illness than controls when followed for a similar duration. Patients with intermittent esotropia were also significantly more likely to have a greater number of mental health disorders, mental health emergency room visits or hospitalizations, and suicidal or homicidal ideation.

**Conclusion** Children with exotropia, unlike those with esotropia, are at an increased risk for developing mental illness by early adulthood. Children with intermittent esotropia appear to be particularly prone to developing significant psychiatric disease by the third decade of life.

**Neural and behavioral mapping of the visual field in true and simulated visual field deficits**

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**Purpose** Our goal was to assess the utility of functional magnetic resonance imaging (fMRI) in simultaneous mapping of perceptual and neural visual field deficits, and in the assessment of neural plasticity processes underlying spontaneous and medication-induced visual field recovery.

**Methods** Neural function of two patients with predominantly unilateral visual field deficits and three volunteers with simulated scotomas was assessed using fMRI retinotopic mapping procedures. In the patients behavioral testing has also been done by asking for reports in a forced choice manner on the color of a 1.66° diameter circle patch pseudo-randomly overlaid on the standard fMRI retinotopic mapping stimuli.

**Results** Retinotopic mapping provided a reliable voxel-based quantification of the extent of both real and simulated visual field deficits. The pattern of BOLD responses in retinotopic visual cortical areas – in particular in the primary visual cortex – showed close correlation with the performance in the perimeter tests, and the behavioral responses obtained during scanning. Moreover, we found a marked difference between true and simulated visual field deficits.

**Conclusion** The presented fMRI retinotopic mapping techniques are suitable for the simultaneous assessment of the functional and behavioral aspects of visual field loss, thus providing an opportunity to investigate neural plasticity in affected patient populations.
**Abstract**

**A case of 2 brothers with congenital horner's syndrome**

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**Purpose** To present a case of two siblings diagnosed with Congenital Horner's Syndrome. The condition developing in related patients has only been previously described once in the literature.

**Methods** The older brother presented at 9 months to his GP with anisocoria. The left pupil appearing smaller than the right. There was no history of birth trauma or any neonatal concerns. The younger sibling was later referred after he was observed to have anisocoria, again the left being the smaller pupil. Both patients were term deliveries with normal labour and no postpartum problems.

**Results** Pharmacological testing with cocaine 10% confirmed the diagnosis of Horner's syndrome. Laboratory and radiological examinations performed by the paediatrician did not reveal any abnormality.

**Conclusion** This case proposes a familial predisposition to the development of Horner's Syndrome and warrants future investigation into the cause.

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**Abstract**

**Harry Moss Traquair (1875-1954) – Scottish ophthalmologist and perimetrst**

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**Purpose** The aim of the study was to: 1. analyze the medical achievements of Harry Moss Traquair and 2. collect all available information concerning his family and private life for a future biography.

**Methods** Typical for the study in the field of the history of science.

**Results** Harry Moss Traquair (1875-1954) was a Scottish ophthalmologist, who devoted most of his professional life to making important contributions to our understanding of visual field studies. His fundamental work *An Introduction to Clinical Perimetry* was for years the primary reference work for perimetry. Traquair carried out many carefully designed experiments concerning different aspects of visual field testing, including working distance, test objects, movement of test objects, illumination, and background. He also contributed detailed recommendations and standards for clinical perimetry in ophthalmology and neurology. He introduced, as well, definitions for some scotomata, which were used for years in the ophthalmic literature. The junction scotoma, often named the Traquair scotoma or the Traquair junction scotoma, is this term used to describe a unilateral temporal visual field defect and is named for the pathology of the ipsilateral optic nerve where it joins the chiasm. Traquair also devoted much work to phenomena which are not considered important any longer, such as haring of the blind spot in glaucoma and visual field defects in tobacco toxic amblyopia. He was an accomplished eye surgeon and had a special interest in dacryocystorhinostomy.

**Conclusion** Traquair was a strong advocate of the benefits of visual field testing in ophthalmology and neurology. But most of all, we will remember him as the scholar who taught that "perimetry is not done by the perimetrst."
Ocular hemodynamic effects of nitrosodilators in healthy subjects

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Purpose Previous studies on the ocular hemodynamic effects of nitrites showed partially contrasting results. The aim of the present study was to investigate the effect of nitrosodilators on choriald and optic nerve head (ONH) blood flow.

Methods The study was performed in a randomized, placebo-controlled, double-masked, 4-way crossover design in 12 healthy male subjects. Nitroglycerine, isosorbide dinitrate or sodium nitroprusside were administered intravenously in stepwise increasing doses on different study days. Physiologic saline solution was used as placebo control. To assess choroidal and ONH microcirculation, laser Doppler flowmetry measurements were performed. Laser interferometric measurements of fundus pulsation were used to assess pulsatile choroidal and ONH blood flow. Ocular perfusion pressure (OFP) was calculated as OPP-2/3MAP-OP. Thereafter a parameter of vascular resistance (R) for each measured blood flow parameter (BF) was calculated as R /OP-BF.

Results Placebo administration did not change any of the measured parameters. We observed small but significant reduction of IOP during the infusion of sodium nitroprusside. The pulsatile choroidal blood flow decreased significantly during the infusion of isosorbide dinitrate (−8.1%) and sodium nitroprusside (−4.3%). The pulsatile ONH blood flow decreased significantly only during the infusion of sodium nitroprusside (−5.7%). In addition, we observed a significant reduction of all calculated parameters of vascular resistance during the infusion of each nitrosodilator (maximal decrease: −16% to −29%).

Conclusion An intravenous infusion of nitroglycerine, isosorbide dinitrate or sodium nitroprusside caused a significant reduction of vascular resistance in the ONH and the choroid.

Minocycline is cytoprotective in human trabecular meshwork cells and optic nerve head astrocytes by increasing expression of XIAP, Survivin and Bcl-2

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Purpose Primary open-angle glaucoma (POAG) is one of the leading cause for blindness. Activation of optic nerve head astrocytes (ONHA) and loss of trabecular meshwork cells (TMC) are pathogenic for this disease. Osmatide stress (OS) and elevated levels of TGF-beta play an important role in the pathogenesis of this neurodegenerative disease. This study investigates possible anti-apoptotic and cytoprotective effects of Minocycline (M) on TMC and ONHA under OS and TGF-beta.

Methods TMC and ONHA were treated with 1µM to 150µM M. Possible toxic effects and IC50 were evaluated after 24h (MTT). To investigate possible protective effects of M on TMC and ONHA, cell proliferation and viability was examined. Expression of XIAP Survivin as well as Bcl-2 and their mRNA was assessed by RT-PCR 24h after treatment with M alone, additional incubation with TGF-beta 2 and OS.

Results M concentrations from 1µM to 75µM showed no toxic effects on TMC and ONHA. Under conditions of OS both TMC and ONHA showed an increase in viability and ability to proliferate when treated with 20-40µM M. RT-PCR yielded an overexpression of XIAP, Survivin and Bcl-2, when TMC or ONHA were treated with 20-40µM M for 24 hours, under OS and when additionally incubated with TGF-beta.

Conclusion We could show that M does not have toxic effects on TMC and ONHA cells up to 75µM. The observed increase in viability and proliferation under OS and TGF-beta2 and the overexpression of XIAP, Survivin and Bcl-2 after treatment with 20-40µM M might prevent apoptotic-celled death in response to cellular stress and may be a protective pathway for TMC and ONHA to avoid progression of glaucomatous degeneration.

Optic nerve reflectivity variations during diffuse lumiance flicker stimulation

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Purpose Neurovascular coupling at the optic nerve is the mechanism that adjusts blood flow in the microcirculation of the optic nerve to support the neural activity induced by vision. Our aim is to assess the variations of the optic nerve reflectivity in the near-infrared during diffuse lumiance flicker stimulation.

Methods One informed and consenting male subject aged 29 was involved in the study so far. A commercial fundus camera (Topcon, Japan) was modified for the purpose of the study. The camera was used to carry out 770 nm reflectivity measurements from the subject’s optic disk and peripapillary region. Neural activity was evoked by diffuse flicker stimulation (2Hz and 12Hz) in the green spectral region (532nm). The measurement protocol consisted of three periods: 20s baseline followed by 60s diffuse flicker stimulation and 20s recovery.

Results During flicker stimulation we observed a decrease in optic nerve reflectance of about 8% at 2Hz and about 6% at 12Hz with respect to the measured baseline.

Conclusion We report a significant decrease in the optic disk and peripapillary reflectance during diffuse visual stimulation at diverse frequencies. These variations could be induced by an increase in blood flow in the microcirculation of the optic nerve. The authors gratefully acknowledge the support of the Fondazione Cassa di Risparmio in Bologna, Italy.

Support for the idea that light is a risk factor in optic neuropathies, like glaucoma

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Purpose Retinal ganglion cell (RGC) axons in the globe contain many mitochondria and it has been hypothesised that light can interact with these organells to affect RGC survival in glaucoma. Studies on different cell types were conducted to support such a proposition.

Methods Near confluent cultures of RGC-5 cells, primary rat retinal cultures, fibroblasts with normal (BRET7) or mitochondria depleted of mtDNA (CHOI) were transferred to incubators containing light (400-760nm; 800-2000 lux; generally 2 days). Some of the cultures were covered with white paper to exclude the light. The cultures were then analysed for cell viability, generation of free radicals (ROS) and for death by apoptosis.

Results Osmatide stress and mitochondrial dehydrogenase activity in retinal cultures (−40-50%), RGC-5 cells (−20%) and BRET7 cells (−30%) was reduced significantly by light. Light reduced the number of GABA-positive neurones (−42-66%) in retinal cultures. Light caused a 3.5 fold increase in TUNEL-positive cells in primary retinal, RGC-5 and BRET7 cultures, than in the dark. ROS staining was also clearly elevated by light. The light-induced toxic effect on the different cell types was significantly blunted by antioxidants like vitamin E and lipic acid. Moreover, light-induced apoptosis was caspase independent but PARP dependent. In contrast, rho0 cells that lacked functional mitochondria were unaffected by light.

Conclusion The present study shows that light can directly affect mitochondrial function to induce apoptosis. This supports the view that light can interact with the many RGC axon mitochondria to affect the viability of GCs and that this may be of significance in the progression of glaucoma.
Effect of induced myopia on scleral mechanics and myofibroblast populations

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Purpose Determine the effect of induced refractive error on a) ocular compliance and b) scleral contractile cell populations in guinea pig.

Methods One week old guinea pigs (n = 29) were monocularly deprived of form vision (MD) for 14 days. Cycloplegic retinoscopy was measured with an IR Optometer. Ocular compliance (change in length/mmHg change in IOP) of MD and control eyes was compared by raising the IOP to 50 mmHg for one hour in 8 anaesthetised animals. A-scan ultrasonic measures of axial length were taken every 10 minutes with raised IOP and after returning IOP to 15 mmHg. Antibodies to DAPI and α-smooth muscle actin (α-SMA) were used to determine the total cell population and cell contractile potential respectively in MD and control sclera.

Results MD induced -4.06 ± 1.88 D of myopia, which was correlated with 169 ± 267 μm of vitreous chamber depth (VCD) elongation (n = 29, R2 = 0.35). Both deprived and control eyes showed negative creep compliance (eye shortening) over one hour of raised IOP equivalent to 1.44 μm shortening per minute. On returning the IOP to 15 mmHg the VCD of the control eye was shorter than baseline by 92.8 ± 88 μm while the deprived eye remained 83.48 ± 37 μm longer than baseline (± SEM) (n = 8). These differences were not statistically significant (p = 0.173). There was however an increase of 6% in the percentage of the total cell population positive for α-SMA in myopic versus control sclera (p = 0.015; n = 7).

Conclusion Myopic and control eyes show similar negative creep compliance in vivo with raised IOP, and no difference in their elastic compliance response. However the α-SMA positive cell population is increased in myopic sclera, suggesting a role in scleral remodelling.

Topical beta-blockers and the risk of cardiovascular mortality

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Purpose Recently, the Blue Mountains Eye Study reported an association between the use of topical timolol and cardiovascular mortality (Lee et al. Ophthalmology 2006). The purpose of the present study was to confirm or falsify this clinically very important finding, using data from the population based Rotterdam Study.

Methods 6971 participants of the Rotterdam Study, a longitudinal population based study of all residents aged 55 years and older from a district of Rotterdam, The Netherlands, were followed from 1990 onwards. Medication use and morbidity were recorded continuously during follow-up. For the current analysis, baseline use of topical beta-blockers and systemic cardiovascular medication as well as baseline cardiovascular morbidity were used, aiming to follow the design of the Blue Mountains Eye Study as close as possible. Cause of death was registered up to 1-1-2005. Data were analysed using Cox regression; Hazard ratios of topical beta-blocker use were adjusted for age, sex, cardiovascular morbidity and use of systemic cardiovascular medication.

Results Mean age at baseline was 69 years (SD 9 years), 146 participants were using topical beta-blockers at baseline. 2726 participants died during follow-up (all cause mortality 40.1%), 611 (9.0%) had a cardiovascular cause of death. Hazard ratio of topical beta-blocker use was 0.80 (95% confidence interval 0.63-1.02, P=0.07) for all cause mortality and 0.78 (0.46-1.29, P=0.32) for cardiovascular mortality.

Conclusion In our data, the use of topical beta-blockers at baseline was not associated with either all cause mortality or cardiovascular mortality during follow-up.
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