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# Ophthalmic Research

**Journal for Research in Experimental and Clinical Ophthalmology**

# EVER 2005

European Association for Vision and Eye Research

## Abstracts

October 5–8, 2005
Vilamoura, Portugal

Guest Editor:
*J. Jonas, Heidelberg, Germany*

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# ABOUT EVER

## EVER 2005

October 5-8, 2005
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EVER is a non-profit organisation. The aims of the Association are to encourage research and the dissemination of knowledge concerning the eye and vision by means of meetings, publications and exchange of information.

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# KEYNOTE LECTURES

EVER 2005

October 5-8, 2005
Vilamoura, Portugal

**1001**

# Evolution of Cataract Surgery, Corneal Transplantation and Refractive Surgery

*Joaquin BARRAQUER*
*Instituto Barraquer, Barcelona*

Although "reclination" of the opaque lens had been used for many centuries, evolution of cataract surgery really starts with Daviel's operation in 1745. However, most advances have been achieved in the 20th Century. The original extracapsular operation of Daviel was substituted by intracapsular extraction of the lens. "Phacoeresis" became the procedure of choice (Ignacio Barraquer,1917). Zonular resistance was a major problem until the advent of "enzymatic zonulolysis" (Joaquín Barraquer, 1958). Implantation of intraocular plastic lenses for correction of aphakia was another important contribution of the past century. Modern extracapsular extraction,including sonophacoaspiration and implantation of injectable posterior chamber lenses lead the way into the 21st century. This was complemented by increased attention to improving the patient's postoperative quality of life (insertion of "pseudoiris" in aniridia, Laser capsulotomy, etc).The challenge to improve the patient's quality of vision lead to the development of safer, less invasive procedures for refractive surgery: Lasik photokeratomileusis, implantation of precrystalline lenses for high myopia ( leaving the "natural" lens in place), etc. Reconstruction of the anterior segment of the eye by combined procedures became possible due to the advances in corneal transplantation: Improvement of instruments and medication to prevent and treat immunologic problems, improved long-term postoperative follow-up, etc.. This also facilitated the performance of large diameter keratoplasties to correct pathological and refractive corneal defects, etc. Excimer laser trephination is another modern option to improve the results. Aspiration of the lens contents through a very small capsulostomy and substitution by an adequate substance to restore accommodation (Phaco-Ersatz, Parel, JM & Barraquer, J.,1980)has not yet passed the experimental stage but may be of great importance for presbycia and aphakia.

The conference will be illustrated by material from the personal casuistics of the author.

1002

# A little bit of light on the lens

*Per SÖDERBERG*
*St. Erik's Eye Hospital, Karolinska Institute, Stockholm*

Radiation from the sun is to a large extent scattered and reflection from the ground may increase the eye exposure. Epidemiological studies have demonstrated that UVR is the most important avoidable risk factor for cortical cataract. The cornea filters out essentially all UVR of wavelengths shorter than 280 nm and the lens almost all the remaining UVR. In vivo exposure of the albino rat eye to UVR induces an exponentially declining increase of light scattering, associated with an increase of sodium and a decrease of potassium, paralleled with a transient increase of water, indicating osmotic swelling. Unscheduled DNA synthesis, p53 expression and apoptosis is observed in the lens epithelium. Swelling of lens epithelial cells and later lens fibers is seen. The spectral sensitivity of the lens for in vivo exposure to UVR, estimated as inverse Maximum Tolerable Dose (MTD), peaks at 300 nm. In vivo UVR around 300 nm is attenuated within 0.5 of the lens surface. In the exposure time domain 5-120 min, the intensity of forward light scattering in the lens, as a function of exposure time at equivalent UVR dose, peaks around 15 min., indicating that the light scattering is a biologically modulated response to the initial photochemical event. The MTD for doses applied fractionated into two equivalent doses, increases exponentially declining, providing a measure of in vivo repair rate and the amount of repair. MTD for avoidance of cataract is strongly species dependent. The sensitivity to UVR decreases exponentially declining with increasing age. Therefore, age should be considered in safety standards for avoidance of UVR induced cataract. Altogether, epidemiological and experimental data provide a basis for improved prevention of the worlds most common cause of blindness, cataract.

**1003**

# Ocular surface regeneration: Present and Future

*Shingeru KINOSHITA*
*Kyoto Prefectural University of Medicine , Ophthalmology, Kyoto*

The purpose of this presentation is to better appreciate a state-of-the-art therapeutic modality and future directions of ocular surface reconstruction via regenerative medicine. Pertinent literature is reviewed, and the data obtained in our basic research and clinical practice is summarized. Briefly, for bilaterally, severely affected ocular surface disorders, two types of transplantable cultivated mucosal epithelial sheets can be used. One is an allogeneic/ autologous corneal epithelial sheet; the other is an autologous oral mucosal epithelial sheet. For this, we established the system of creating cultivated corneal or oral mucosal epithelial stem cell sheets by co-culturing with 3T3 fibroblast and air-lifting on amniotic membrane. Over 50 severe ocular surface disorders received these cultivated mucosal epithelial sheets, for either corneal surface reconstruction or fornical conjunctival reconstruction. During follow-up (maximum; 66 months), the ocular surfaces were well restored, though there have been biological and/or immunological epithelial troubles in various degrees, postoperatively. In conclusion, the transplantation of cultivated mucosal epithelial stem cell sheet is a cutting-edge surgical modality for severe ocular surface disorders, though several technical improvements will still be necessary in the future.

**1004**

# The Spark of Life? A Fundamental Role for Electric Fields in Guiding Cell Behaviour

*John FORRESTER*
*University of Aberdeen, Institute of Medical Sciences, Ophthalmology, Aberdeen*

The forces that guide cell behaviour during development, embryogenesis, differentiation, wound healing, and responses to invasion by foreign or tumour antigens are of considerable interest since they determine the outcome of many of these processes. One of the longest recognised forces relates to the presence of endogenous electric fields, generated in tissues and cells by partitioning of electrolytes and large charged molecules. Electric fields underpin many ocular physiological and pathological processes and study of these processes has shed light on general mechanisms of cell behaviour. Some of these phenomena will be illustrated in this presentation in the context of the eye.

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# The Cause and Prevention of Nuclear Cataracts: Oxygen and the Vitreous Body

*David BEEBE (1, 2), SHUI Y-B (1), HOLEKAMP N M (1, 3), HAROCOPOS G J(1) , KRAMER B (1)*
*(1) Ophthalmology and Visual Sciences, St. Louis, Missouri*
*(2) Cell Biol. Physiol., St. Louis, Missouri*
*(3) Barnes Retina Institute*

**Purpose** Nuclear cataracts are associated with oxidation of the proteins and lipids in the center of the lens. Hyperbaric oxygen treatment or vitrectomy promotes the formation of nuclear sclerotic cataracts. We tested whether degeneration of the vitreous body was a risk factor for age-related cataracts and whether vitrectomy increased exposure of the lens to oxygen.

**Methods** In eye bank eyes, vitreous body degeneration was assessed by measuring the fraction of the vitreous body that was liquid. Cataract was quantified by slit lamp examination of isolated lenses. An optical oxygen sensor (optode) was used to measure oxygen levels in consenting patients before and after vitrectomy and in postmortem samples of vitreous humor.

**Results** After adjusting for age, degeneration of the vitreous body was associated with increased risk of nuclear ($p<0.0001$), but not cortical or posterior subcapsular opacities. Oxygen levels in the human vitreous body were low (<1.5%). Vitrectomy was associated with an acute, eight-fold increase in the exposure of the lens to oxygen. Months after vitrectomy, the level of oxygen near the posterior of the lens was still increased by ~30% ($p<0.02$). Diabetics had lower levels of oxygen in the vitreous body than non-diabetics. Human vitreous consumed oxygen in an ascorbate-dependent manner.

**Conclusions** The vitreous body protects against nuclear cataracts by maintaining low levels of oxygen at the posterior of the lens. Lower oxygen levels in the vitreous body may explain why diabetics appear to have lower rates of post-vitrectomy and age-related nuclear cataracts than non-diabetics. The gel structure and metabolic activity of the vitreous body reduce the exposure of the lens to oxygen from the retina.

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# Assessment of vision - Monitoring impairments and predicting functional abilities

*Ian BAILEY*
*School of Optometry, University of California, Berkeley*

**Purpose** To show that for both monitoring and predicting functional abilities, clinical tests of vision should have narrow confidence limits for change and that some measures of performance should be made at supra-threshold stimulus levels.

**Methods** Comparisons between test and retest results for both visual acuity measures and clinical grading scores show tests to be very insensitive to change when there is a high rate of perfect agreement between test and retest scores.
We compare critical angular size (smallest size for maximum efficiency) or preferred angular size (angular size adopted when reading small print) as alternative methods for determining the print size that is optimal for reading.
For contrast sensitivity, new computer-based tests measure response times to low contrast stimuli during detection tasks and in search tasks and we show relationships between target contrast and performance efficiency for normally and partially sighted subjects.
**Results** Comparisons between test and retest for visual acuity measures and for clinical grading scores show tests are insensitive to change when there is a high rate of perfect test/retest agreement.
We compare critical angular size (smallest size for maximum efficiency) or preferred angular size (angular size adopted when reading small print) as alternative methods for determining the optimal print size.
New computer-based contrast sensitivity tests measure response times to low contrast stimuli during detection tasks and search tasks and they show relationships between target contrast and performance efficiency.

**Conclusions** Confidence limits for change depend on the fineness of the scale. Relationships between response efficiency and stimulus strength can be useful in predicting functional abilities.

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# Medical Therapy of Glaucoma in the Future: From Cell Culture to Clinic

*Paul KAUFMAN*
*Ophthalmology and Visual Sciences, Madison*

**Purpose** To review some of the most important new findings related to glaucoma therapy and related research, and where we they will take us clinically.

**Methods** Current "on – the –edge" research will be summarized and derivative future directions proposed.

**Results** Enhancing outflow through the trabecular meshwork will be accomplished with agents that alter the cytoskeleton and interactions with the extracellular matrix. Uveoscleral outflow enhancement will continue to be targeted as the major regulatory pathways are further clarified. Retinal ganglion cell neuroprotection, rescue and replacement strategies, via pathways now being elucidated, must be implemented early on and must address the central changes that also occur. Overexpression or suppression of proteins involved in regulating these processes may be delivered by gene therapy, and other novel ocular drug delivery strategies for the anterior and posterior segment. Methods for continuous monitoring of IOP as well as detection of functional and structural changes in the retina and centrally will be brought into play.

**Conclusions** Therapies for the future will emphasize outflow enhancement, and neuroprotection, rescue and perhaps replacement. New modalities for therapeutic delivery will allow sustained exposure and action. Innovations in detection and monitoring of disease and therapy-induced structural and functional effects at both the tissue and cellular level will advance and come on line.

# POSTERS

EVER 2005

October 5-8, 2005
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## An experimental model to measure real intraocular pressure (IOP) in LASIK

*HERNANDEZ VERDEJO JL (1, 2), BOLíVAR G (3), ROMAN JM (1), TORRES J (1), TELIS MA (1, 4)*
*(1) VISSUM Hospital Oftalmológico Madrid, Madrid*
*(2) Universidad Complutense de Madrid, Madrid*
*(3) Hospital Universitario Príncipe de Asturias, Madrid*
*(4) Universidad de Alcalá, Madrid*

**Purpose** To design a model to measure real IOP during lasik in pig eyes.

**Methods** A reusable blood pressure transducer was used. Each eye was placed on a stand with sufficient support for the surgical procedure. The globes were inflated with 5% glucosated solution through the optic nerve to attain an IOP of 10-20 mmHg. At that moment, a 27-gauge catheter was inserted from pars plana to the anterior chamber cavity. Real IOP was transmited via the catheter liquid column to the external sensor. The suction ring was applied and a flap was created. During the procedure, IOP was registered with the ML110 Bridge Amplifier connected to the baro transducer.

**Results** Values of real IOP was mesured in freshly enucleated pig eyes during the lasik procedure. The model was able to register instantaneously the changes in IOP induced by the application of a suction ring and the creation of a flap in pig eyes with good reproductibility. At the end of the procedure the IOP values remained stable.

**Conclusion** We have developed an experimental model to observe the course of suctioning and the changes in IOP simultaneously. This model seems to be accurate to obtain IOP values from the anterior chamber cavity during lasik.

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## Ocular and Dermal Hydration: The Release of Natural Moisturising Agents from Hydrogels

*TIGHE B, BAHIA R*
*Biomaterials Research Unit, Birmingham*

**Purpose** To investigate the release of natural moisturising factors from monophasic and biphasic hydrogels which have the potential to counteract unacceptable symptoms of dryness associated with the eye or skin.

**Methods** Monophasic hydrogels were loaded with 2-pyrrolidone-5-carboxylic acid (PCA) and biphasic gels were loaded with either PCA or Vitamin E. The active agents were released into either an aqueous substrate or a lipoidal substrate. The actives were extracted from the substrates and UV spectroscopy was used to calculate the concentration of the actives released.

**Results** Release of a component from a polymer matrix into another phase is governed by its size, charge, structural affinity and octanol water-partition coefficient. This study illustrates that it is possible to release hydrophilic/ hydrophobic actives from a biphasic hydrogel through an aqueous/ lipoidal route respectively with release profiles adjusted to give the required delivery characteristics. Vitamin E can be released from biphasic hydrogels into the lipid layer of the eye to replace lipid solid antioxidants adsorbed into the lens.

**Conclusion** The aqueous environment dominates the eye and the lipoidal environment dominates the skin. Breakdown in these environments and control mechanisms results in dry skin or dry eye symptomology. Early stages of dry eye in particular contact lens induced can be resolved by the delivery of selected natural moisturising factors. The use of a contact lens as a delivery matrix offers great potential in this respect provided that subtleties of the release mechanisms are properly understood.

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## Femtosecond Laser Energy Thresholds for Surgical Use in Corneal Tissue

*VOSSMERBAEUMER U (1), JONAS JB (2)*
*(1) Dept. of Ophthalmology / University Eye Hospital, University of Heidelberg, Faculty of Clinical Medicine, Mannheim*
*(2) Dept. of Ophthalmology / University Eye Hospital, University of Heidelberg, Faculty of Clinical Medicine, Heidelberg*

**Purpose** To examine the energy dependence of near-infrared intracorneal femtosecond(fs)laser incisions.

**Methods** Fs-laser procedures were performed in 25 freshly enucleated porcine eyes and in 20 human corneal donor eyes. Specimen were histomorphometrically examined.

**Results** Size of the intrastromal effects ("bubbles") ranged between 10µm and 100µm in diameter. Independently of the pulse focus depth, bubble size was statistically independent ($p>0.10$) from the energy applied. In oblique effect patterns, tissue bridges could usually be found.

**Conclusion** fs-laser incisions not parallel to the corneal surface may not lead to a contiguous separation of the tissue. Within a range of 2 to 15 µJ, intracorneal bubble size is not strongly associated with the energy applied. Pulse Energy within variation corridors of 2 to 15 µJ may not be the main influencing factor in corneal tissue disruption quality of femtosecond laser pulses.

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## Effectiveness & safety of Astigmatic Keratotomy for general ophthalmologist

*ARIF FA, MORIARTY AP*
*Ophthalmology/ Stepping Hill Hospital, Stockport*

**Purpose** To study efficacy of Astigmatic Keratotomy to manage astigmatism for patients performed either during phacoemulsification or as a separate procedure following anterior segment surgery.

**Methods** Astigmatic Keratotomy is an incision in the steep meridian of the cornea increasing the radius of the corneal curvature (wound gape) in that meridian, flattening it and reducing refractive power.27 eyes of different patients had arcuate incisions to manage astigmatism.The Lindstrom nomogram was used to predict or case series. Examination before surgery included visual acuity with and without correction, computer assisted corneal topography, ultrasound pachymetry, complete ocular examination. Prior to astigmatic keratotomy, the cornea and ocular surface should be healthy, keratoconjuctivitis sicca and blepharitis must recognized and treated.The incisions were made with a front cutting motion of the triple edge diamond knife. Lindstrom's nomogram was used to calculate the number of incisions and arc length.

**Results** Significant correction in astigmatism (Cylinder power) and a dramatic improvement in aided visual acuity. The mean preoperative cylinder power was 3.9D with a range from (-6.10D - +7.0D). The mean post operative cylinder power was 1.8D with a range from (-3.75D - +4.0D).

**Conclusion** Astigmatic surgery should be based on keratometry and computer corneal topography rather than manifest refraction. It is a very safe procedure to manage astigmatism. Our results confirm that this can be performed by the general ophthalmologists.

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## Mycophenolic Acid Modifies Fibrotic Responses by Human Tenon Fibroblasts in Vitro

*AMER RA (1), PLIXEDDU IP (2), ANTEBY IA (1), LEVI-SCHAFFER FL-S (2), SOLOMON AS (1)*
*(1) Ophthalmology Department-Hadassah University Hospital, Jerusalem*
*(2) Department of Pharmacology, School of Pharmacy, Faculty of Medicine, Jerusalem*

**Purpose** To evaluate the effects of mycophenolic acid (MPA) on human tenon fibroblast proliferation, apoptosis and necrosis in vitro

**Methods** Human tenon fibroblasts were obtained from tissue explants from young healthy patients during strabismus surgery. Proliferation of subconfluent fibroblasts was assessed by using the ($^3$H) thymidine-incorporation assay. Fibroblast proliferation was assessed following TGF-beta1 stimulation at the optimal concentration of 10 ng/ml. MPA was added (10-3 M to 10-8 M in DMEM with 2% fetal calf serum) to the cultures for 24 hours. Fibroblast apoptosis and necrosis induced by MPA was assessed by the addition of annexin-V and propidium iodide stains, and by evaluating positively stained cell populations with FACS

**Results** MPA significantly inhibited TGF-beta1-induced fibroblast proliferation with optimal concentrations ranging from 10-3 to 10-6 M. The addition of MPA at 10-3 M suppressed TGF-beta1-induced fibroblast proliferation by 89.9% ($p<0.001$). MPA did not cause apoptosis nor did it induce significant necrosis of the fibroblast populations under study

**Conclusion** Mycophenolic acid demonstrated a significant antiproliferative effect on human tenon fibroblasts in vitro. This effect is not the result of cytotoxic or apoptotic effect of the drug. This effect can be utilized to modify fibrotic responses of the ocular surface in various cicatrizing and allergic disorders, as well as in postoperative fibrosis in pterygium excision and in glaucoma filtering surgey

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## Proliferative capacity and cytokeratin expression in the abberant endothelium of posterior polymorphous corneal dystrophy

*MERJAVá S, JIRSOVA K, VESELA V, MARTINCOVA R, LISKOVA P, FILIPEC M*
*Laboratory and Ocular Tissue Bank, Dept of Ophthalmology, University Teaching Hospital and Charles University, Prague*

**Purpose** To assess the cytokeratin (CK) spectrum of cells present in the posterior corneal layer of tissues obtained from posterior polymorphous corneal dystrophy (PPCD) patients from the Czech Republic.

**Methods** Twelve corneal buttons and one specimen of trabecular meshwork (TM) from PPCD patients that underwent graft or glaucoma surgery, five corneal buttons and two TM specimens obtained from healthy donors were used. Indirect immunofluoresce was performed using antibodies against CK1/10/11, CK3/12, CK4, CK5/6, CK7, CK14, CK16, CK18, and CK19. The number of positive cells was scored under a fluorescent microscope using a scale from N0 to N4 (N0: 0%, N1: 1-25%, N2: 26-50%, N3: 51-75%, N4: 76-100%). The intensity of the signal was scored as follows: 0 – no staining, 1 – mild, 2 – moderate, 3 – intense, and 4 – very intense staining.

**Results** The abnormal PPCD endothelial cells showed strong reactivity to CK7, CK18, CK19, and CK3/12, scattered weaker positivity to CK4, CK5/6, CK14 and CK16. PPCD endothelium was completely negative for cytokeratins 1,10/11. The surface cells of TM were positive for CK 7, 18 and 19. The endothelium and TM of all control specimens were negative for all tested cytokeratins. The PPCD and control epithelium revealed practically the same staining pattern.

**Conclusion** We demonstrated that the abnormal endothelium of PPCD patients express not only cytokeratins that are abundant (CK3/12) or weakly present (CK4, 14) in control corneal epithelium, but also cytokeratins (CK 7, 18, 19) which were not usually detected in healthy corneal epithelium. By its CK composition the aberrant PPCD endothelium share features of both simple and squamous stratified epithelium.

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## The depletion of macrophages and/or CD4+ cells prolongs corneal xenograft survival

*VITOVA A (1, 2), PINDJAKOVA J (1), FILIPEC M (2), JIRSOVA K (2), ZAJICOVA A (1), HOLAN V (1)*
*(1) Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague*
*(2) Department of Ophthalmology, 1st Medical Faculty and General Teaching Hospital, Prague*

**Purpose** To investigate the influence of local depletion of macrophages alone or in combination with systemic depletion of CD4+ or CD8+ cells on corneal xenograft (XGR) survival and on the production of Th1 and Th2 cytokines and nitric oxide (NO) in rejected corneal XGRs.

**Methods** Xenogeneic (Lewis rat to BALB/c mouse) orthotopic corneal transplantation was performed. Recipients were treated with subconjunctivally administered liposomes containing dichlormethylen diphosphonate (clodronate-LIP) and/or with monoclonal antibody (mAb) anti-CD4 or anti-CD8 applied intraperitoneally. Expression of genes for inducible nitric oxide synthase (iNOS) and for cytokines (IL-2, IFN-γ, IL-4 and IL-10) was determined in rejected corneal XGRs by RT-PCR. Production of cytokines and NO by cultivated rejected graft explants was measured by ELISA and by the Griess reaction, respectively.

**Results** Treatment of corneal XGR recipients with mAb anti-CD4 or clodronate-LIP significantly prolonged corneal XGR survival but treatment with mAb anti-CD4 was superior. Depletion of CD8+ cells did not significantly prolong survival of corneal XGRs. Expression of genes for cytokines IL-2, IL-4 and IL-10 was suppressed in corneal XGRs from recipients treated with mAb anti-CD4. In all recipients treated with clodronate-LIP inhibition of the expression of the gene for iNOS was observed. Expression of the gene for IFN-γ and production of cytokine IFN-γ was present in all rejected corneal XGRs of all groups of recipients.

**Conclusion** CD4+ T cells and macrophages play the important role in corneal XGR rejection. Rejection of corneal XGRs is further associated with the production of cytokine IFN-γ.

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## Flurbiprofen decreases the gene expression of TNF-α in rabbit corneas exposed to artificial sunlight in vivo

*KARAMPATAKIS VE (1), BASLI E (2), GOULAS A (3), VARSAMIDIS K (4), KOMNENOU A (5), KAROUTIS A (1), KOKKAS B (3), MIRTSOU-FIDANI V (3)*
*(1) Laboratory of Experimental Ophthalmology, Aristotle University of Thessaloniki, Thessaloniki*
*(2) Ophthalmology, Aristotle University of Thessaloniki, Thessaloniki*
*(3) Pharmacology, Aristotle University of Thessaloniki, Thessaloniki*
*(4) Physiology, Technological Educational Institution of Thessaloniki, Thessaloniki*
*(5) Clinic of Surgery, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki*

**Purpose** The purpose of the present study was to test the ability of topically applied flurbiprofen, a well-known non-steroidal anti-inflammatory agent and cyclooxygenase inhibitor, to repress TNF-α expression in rabbit corneas, in vivo, under conditions simulating over-exposure to natural sunlight.

**Methods** A specifically designed set-up was used to irradiate both eyes of immobilized albino rabbits (n = 7) with a 300 W sunlamp, for a total time period of 30 minutes. In each animal, one eye was treated with standard doses of sodium flurbiprofen (0.03%) eye drops before, as well as after irradiation, while the other eye was left untreated. 24 hours following irradiation both corneas were excised and analysis of TNF-α expression was assessed with a semi-quantitative RT-PCR method using appropriate primers and reaction conditions. Analysis of GAPDH expression was used as internal reference. Paired samples Student's t-test was used for comparison of relative TNF-α expression between flurbiprofen-treated and untreated eyes.

**Results** Local administration of sodium flurbiprofen under the conditions used in this study, appears to be effective in decreasing relative corneal TNF-α expression by almost half ($P = 0.027$) in rabbit eyes exposed to the radiation.

**Conclusion** Our study provides indirect in vivo evidence that TNF-α expression in rabbit corneas exposed to radiation emitted by a sunlamp is related to cyclooxygenase activity, and points to the possible use of topically applied cyclooxygenase inhibitors as a means of preventing corneal inflammation induced by over exposure to sunlight.

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## Advantages of Dacrio TC versus Traditional Dacriocistography

*PESCE G (1), MESIANI MAZZACUVA F (1), CATALANO C (1), CARNOVALE SCALZO G (1), SCORCIA V (1), BRUZZICHESSI D (1), PARDATKSCHER K (2), SCORCIA G (3)*
*(1) Eye Clinic, University of Catanzaro "Magna Graecia", Catanzaro*
*(2) Neuroradiology,University of Catanzaro "Magna Graecia", Catanzaro*
*(3) University of Catanzaro "Magna Graecia", Catanzaro*

**Purpose** This study examines the advantages of the dacrio-TC versus the traditional dacriocistography

**Methods** 2 groups of 50 patients ( 57 women and 43 men, mean age 50 years) with epiphora for low lacrimal tract obstruction were recruited and successively each group were submitted to dacrio-TC and dacriocistography

**Results** the study was showed that the dacrioTC versus dacriocistography is a good diagnostic tool to dynamic evaluation of lacrimal tracts.

**Conclusion** Advantages: - Facility execution - Greater compliance - Quality better radiological images - processing and analysis of the images and relationships with the surrounding bony structures. - Possibility of control in the follow-up of the DCR.

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## Within and Between Session Repeatability of Topographic Data Using Medmont E-300 Corneal Topographer

*FERNANDES P, GONZALEZ-MEIJOME JM, QUEIROS A, JORGE J, DIAZ-REY A, ALMEIDA JB*
*Physics (Optometry), Braga*

**Purpose** The aim of the present study was to analyze within- and between-session repeatability of topographic corneal parameters.

**Methods** Sixty eyes from thirty young adults where evaluated. Nine locations including corneal center, 4 locations at 1,5 mm and 4 locations at 3,5 mm beyond the corneal center were examined on three separate sessions, taken three repeated measurements each time.

**Results** Mean values and coefficients of variability within and between sessions are reported for central and peripheral axial curvature and elevation topography as well as other topographical indices including eccentricity, BFS, I-S, SAI and SRI. At center, each individual measurement of axial curvature within same session was not different from the mean value (mean diff.<0.03D; p>0.05; r2>0.99). The same occurred between sessions (mean diff.<0.025D; p>0.05; r2>0.98). Within-session differences at periphery were smaller than 0.05D (range 0.01 to 0.04D) and between different sessions (range 0.02 to 0.09D). Only differences for the most peripheral superior location exceeded 0. 1D. Elevation data, measured in microns followed a similar behaviour. Regarding other topographic parameters, differences among different sessions were statistically significant for SRI (mean diff.=0.032; r2<0.50) and SAI (mean diff.=0.078; r2<0.65). Eccentricity in the steep meridian displayed higher variability than in the flat one.

**Conclusion** Present results are relevant to estimate the limits of normality of these values on normal corneas, as well as their degree of intra- and inter-session variability not only at corneal center but over the whole cornea, particularly in longitudinal studies.

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## Traumatic wound dehiscence after penetrating keratoplasty

*WILLIAMS MA, GAWLEY SD, FRAZER DG*
*Ophthalmology, Belfast*

**Purpose** To present our experience of traumatic wound dehiscence after penetrating keratoplasty

**Methods** Cases were identified and data recorded by retrospective case note review. The cases were analysed in terms of age, original indication for the corneal graft, nature of trauma, time since the graft, presence of sutures, nature of ocular injury sustained, surgery required and final visual acuity compared with best visual acuity before the trauma.

**Results** Fourteen patients were identified who had experienced traumatic wound dehiscence after penetrating keratoplasty. There was a bimodal distribution of ages of patients; young and elderly. The young group was predominantly male. The aetiology followed a corresponding bimodal distribution, assault being more common in the young and falls in the elderly patients. Final visual outcomes varied widely.

**Conclusion** Penetrating keratoplasty wounds are vulnerable to injury even years after the graft operation, whether or not sutures are still present. Patients should be advised of this, and try to avoid situations where their eye may be at risk of injury. Counseling is needed prior to penetrating keratoplasty to explain the lifetime risk. Visual outcomes after surgical repair vary, depending primarily on the extent of other ocular injury occurring alongside corneal wound dehiscence.

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## Validity of Nelson's Classification in the long term follow-up of ocular burns

*GICQUEL JJ (1), BALAYRE S (1), MILINCHIEVITCH S (2), MERCIE M (1), DIGHIERO PLD (1)*
*(1) Ophthalmology / CHU Jean Bernard, Poitiers*
*(2) Pathology / CHU Jean Bernard, Poitiers*

**Purpose** To compare Nelson's classification to direct immunofluorescence, and flow cytomtry on specimens obtained by impression cytology, during a 2 years follow-up of severe ocular burns.

**Methods** During 2 years, we used impression cytology in the follow up of 35 patients with Grade II to VI ocular burns treated with AMT. Impressions cytology samples were obtained with Supor® filters (Gelman / Ann Harbor) before treatment on patient arrival, and during the follow up (Day 5, 10, 30, 2 months, 6 months, 1 year, 2 years). Samples are stained with PAS (Periodic Acid Schiff), Alcian blue (for Nelson's Classification) and studied in direct immunofluorescence as well as flow cytometry.

**Results** CMH class II (HLA-DR) (that constitutes a major eye surface inflammation marker) antigenic expression study gives the prognosis of severe ocular burns.There is an accurate correlation bettween Nelson's classification and immunostaining.Both Nelson and immunostaining reveal a persisting level of inflammation, even though slit lamp examination shows non inflammatory ocular surface.According to Nelson's classification patients with a Grade III at Day 30 have a poor prognosis.

**Conclusion** Severe ocular burns prognosis has been changed thanks to AMT. Nelson's Classification gives good results in the follow-up of ocular burns.It's results correlate well with immunostaining and flow cytometry data obtained from impression cytology specimens.

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## Morphological changes in optic nerve head evaluated with oct before and after LASIK surgery

*ARRANZ E (1, 2), ROMAN JM (1), SÁNCHEZ JM (1), MARINA C (1), TELIS MA (1, 2, 3)*
*(1) Vissum Hospital Oftalmológico de Madrid, Madrid*
*(2) Hospital Universitario Príncipe de Asturias, Alcalá de Henares*
*(3) Universidad de Alcalá, Alcalá de Henares*

**Purpose** To evaluate changes occurred with LASIK in optic nerve head measured with optical coherence tomography (OCT).

**Methods** This a pilot study. We have selected 20 eyes of ten patients who had myopic LASIK and were operated with M2 microkeratome carrying the instructions in the selection of the rings, stoppers, and heads attending the keratometry values of the eyes.We have done OCT preop, one and three months postop.

**Results** 20 LASIK operated eyes has been evaluated with OCT and we have measured the changes found in optic nerve head.The sex distribution was: 9 men and 11 women, the mean age was: 33.29 +- 6.65 years and the mean spherical equivalent were: -3.113 +- 1.25 dioptries. The mean intraoperative suction time was: 18 +- 3.5 seconds. The parameters evaluated were: Vertical integrated rim area, horizontal integrated rim width, disk area, cup area, rim area, cup/disk area ratio, cup/disk horizontal ratio and cup/disk vertical ratio.We have found a statistical significant difference in disk area: 2.179 +- 0.388 mm2 preop vs 2.242 +- 0.386 mm2 at month (p=0.04) and in cup area: 0.404 +- 0.268 mm2 preop vs 0.437 +- 0.258 mm2 at month (p=0.02). In the rest of parameters have no significant differences.

**Conclusion** These results suggest that the increase of IOP during LASIK could affect optic nerve.

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## The role of Hyaluronic acid in ocular and joint lubrication

*RASUL N, TIGHE B*
*Biomaterials Research Unit, Birmingham*

**Purpose** To study the role of Hyaluronic acid (HA) in ocular and joint lubrication and to evaluate the in-vitro activity of HA from different biological sources.

**Methods** Solutions of HA (in 0.1M Phosphate Buffer solution) from Rooster comb, human umbilical cord, streptococcus zooepidemicus and the vitreous humour were evaluated. The primary structure and the purity of the different HA sources were deduced from Nuclear Magnetic Resonance (NMR) Spectroscopy. Rheological studies were carried out on a Bohlin CV50 rheometer. The viscosity of the solutions was measured as a function of shear stress using a CP10/20 mm plate at 310K. Frictional behaviour was studied on a CSM Nanoscratch tribometer (load of 60 mN, speed 30 mm/min) using a sliding contact lens (1-day Acuvue) with the HA solution under study over a sliding distance of 20mm with a polyethylene terephthalate substrate.

**Results** 1H-NMR spectra revealed the primary structure of the HA sources and confirmed thier purity. Rheology analysis suggests that HA solutions (0.4% w/v) exhibit non-Newtonian, and shear thinning (pseudoplastic) behaviour. The mean coefficient of friction for each HA solution under study was calculated. These values seemed to be dependent on the HA source.

**Conclusion** HA solutions exhibit viscoelastic behaviour with HA from the rooster comb showing the highest molecular weight. Frictional analysis indicates that the source of HA affects its lubricating ability. HA from the umbilical cord showed the lowest mean coefficient of friction and appears to be much lower than the mean coefficient of friction obtained with conventional HA ophthalmic comfort drops for dry eye.

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## The effect of wearing time in refractive index of conventional and silicone hydrogel contact lenses: a comparative study

*LIRA M (1), OLIVEIRA M (1), VILAR EP (2)*
*(1) Physics, Braga*
*(2) Optics and Optometry, Santiago de Compostela*

**Purpose** The aim of this study was to measure refractive index before and after contact lens wear and compare the results between the 4 silicone hydrogel and a disposable hydrogel contact lenses

**Methods** The measurements were performed on 72 contact lenses used in a daily wear schedule, with various refractive indexes and different water contents. 14 Acuvue®Advance™with Hydraclear, 11 O2Optix™, 12 Focus® Night & Day™, 8 Purevision™ and 27 Acuvue®. The mean age of the patients was 22.1±4.2 yearsEach lens was measured at two times: before being weared and after 1 month for silicone hydrogel lenses and 15 days for the hydrogel lens. The refractive index considered for each lens was the average of 5 consecutive measurements obtained with an automated refractometer (CLR 12-70)

**Results** The mean value (±SD) of refractive index for the new contact lenses is 1.398(±0.008) for Acuvue®, 1.408(±0.003) for Acuvue®Advance™with Hydraclear, 1.420(±0.010) for Purevision™, 1.426(±0.010) for Focus® Night & Day™, and 1.422(±0.010) for O2Optix™. The mean values (±SD) of refractive index for the same lenses respectively, after being wear were 1.410(±0.002), 1.409(±0.002), 1.420(±0.010), 1.427(±0.010) and 1.422(±0.001)It means, that only for Acuvue, the difference is statistical significant for the refractive index measures with p<0.01

**Conclusion** There are no significant differences between the measurements obtained in refractive index before and after contact lenses wear for the silicone hydrogel contact lenses.The conventional hydrogel, the one with higher water content, so more prone dehydration, are the only lens that has significant differences in refractive index after being wear

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## Pre-lens tear film stability before and after contact lenses wear

*LIRA M (1), OLIVEIRA ME (1), VILAR EP (2), SANTOS L (3), AZEREDO J (3)*
*(1) Physics, Braga*
*(2) Optics and Optometry, Santiago de Compostela*
*(3) Biological Engineer, Braga*

**Purpose** The purpose of this study was to observe and compare the pre-lens tear film (PLTF) of silicone hydrogel lenses and a conventional disposable hydrogel lens worn in a daily wear for 30 days and 15 days respectively.

**Methods** - The Non-Invasive Tear Break-Up Time (NITBUT) was measured on 31 eyes: 6 Galyfilcon A, 5 Balafilcon A, 5 Lotrafilcon A, 5 Lotrafilcon B and 10 Etafilcon A. The mean age of the patients was 23.6 ± 5.5 years. All lenses were used in a daily wear schedule.- When contact lenses are being worn, the pre-lens tear film non-invasive break-up time (PLTF NITBUT) is recorded.- The Keeler Tearscope Plus, with the help of a grid insert, was used to observe the regularity of the image of the grid. It was measured the time interval between the last blink and the appearance of the first distortion on the grid.- The pre-lens tear film was evaluated for 3 times in the first day of use of each lens and after 30 days (silicone hydrogel lens) and 15 days (conventional hydrogel) of wear.

**Results** - The mean value (± SD) of PLTF NITBUT for the new contact lenses was 6.21s (±2.44) and after wear was 5.44s (±2.38). - If we consider each material separately, we have the mean value (±SD) of the new contact lenses and the mean value (± SD) for weared lenses respectively: o Galyfilcon A : 5.79s (±2.39), 4.59s (±2.11)o Balafilcon A : 5.63s (±1.53), 7.03s (± 2.16)o Lotrafilcon A: 8.40s (±2.77), 5.83s (±3.41)o Lotrafilcon B: 7.24s (±3.17), 3.84s (±1.00)o Etafilcon A : 5.13s (±1.72), 5.77s (±2.28)

**Conclusion** - In this study there are no significant differences between the measurements obtained for pre-lens tear film stability before and after contact lenses wear.

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## Effects of biglycan on keratocytes proliferation

*KOULIKOVSKA M, PODSKOCHY A, FAGERHOLM PPP*
*Linköping University, Linköping*

**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 envolvement of biglycan in the corneal repair process. In the present study, we have investigated possible involvement of biglycan in the modulation of the proliferation of native and activated keratocytes.

**Methods** To assess the effect of biglycan on keratocyte proliferation, serial dilutions of keratocytes were cultured under serum-free conditions in the presence or absence of biglycan. Alternatively, keratocytes treated with FBS or TGF-beta were used. Subsequently, keratocyte proliferation was quantified by CyQuant Cell Proliferation Assay Kit.

**Results** In resting keratocytes proliferation was significantly reduced by biglycan. Coincubation with FBS and biglycan significantly reduced the proliferation rate of keratocytes. Incubation with TGF-beta resulted in significantly reduced proliferation of keratocytes, which was reduced in the presence of biglycan although the latter reduction was not statistically significant.

**Conclusion** In native keratocytes proliferation was inhibited by biglycan. More importantly, biglycan counteracted the effects of growth factors being present in fetal bovine serum. Therefore, it is tempting to speculate that biglycan might be an important factor for regulating the proliferation during corneal wound healing. Further studies, however, are needed to elucidate those pathways by which biglycan exerts its antiproliferative effects in keratocytes.

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## Refractive Index and Water Content of Conventional and Silicone Hydrogel Contact Lenses

*GONZALEZ-MEIJOME JM (1), LOPEZ-ALEMANY A (2, 3), LIRA M (1), ALMEIDA JB (4), PARAFITA MA (5)*
*(1) Physics (Optometry) - University of Minho, Braga*
*(2) Optics - Univerity of Valencia, Valencia*
*(3) Institute of Ophthalmology and Optometry of La Costera (IOOC), Novetle*
*(4) Physics - University of Minho, Braga*
*(5) Surgery (Ophthalmology) - University of Santiago de Compostela, Santiago de Compostela*

**Purpose** The purpose of the present study was to measure equilibrium water content and refractive index of conventional and silicone hydrogel soft contact lenses using a hand refractometer and an automated refractometer.

**Methods** Sixteen soft contact lenses were used in this study including twelve conventional soft contact lenses not containing siloxane moieties (water content range: 38,6-74%) and the four silicone hydrogel based contact lenses currently available (water content range: 24-36%). The Atago N-2E hand refractometer and the CLR 12-70 digital refractometer were used.

**Results** Measured equilibrium water content and refractive index correlate better when measured with the instruments used in this study (r2=0,979, p<0,001) than nominal parameters (r2=0,666, p<0,001). The linear relationship that correlates nominal and measured equilibrium water content shows higher spread of data when considered all lenses (r2=0,840) than when conventional hydrogel (r2=0,953) and silicone hydrogel contact lenses (r2=0,967) are analyzed separately. Regarding refractive index, the relationship between nominal and measured values when all the lenses are considered together (r2=0,794) becomes weaker when conventional hydrogel are considered separately (r2=0,688), while a stronger relationship is observed for silicone hydrogel lenses (r2=0,939). Manual refractometry overestimates equilibrium water content of silicone hydrogels.

**Conclusion** New relationships are presented that correlate nominal and measured values of water content and refractive index for the silicone containing hydrogels. Present results are of interest to plan clinical studies involving the measurement of equilibrium water content of current hydrogels.

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## Evaluation of Brix Scales to Correlate Refractive Index and Water Content of Current Conventional and Silicone Hydrogel Contact Lenses

*GONZALEZ-MEIJOME JM (1), LOPEZ-ALEMANY A (2, 3), LIRA M (1), ALMEIDA JB (4), PARAFITA MA (5)*
*(1) Physics (Optometry) - University of Minho, Braga*
*(2) Optics - University of Valencia, Valencia*
*(3) Institute of Ophthalmology and Optometry of La Costera (IOOC), Novetlé*
*(4) Physics - University of Minho, Braga*
*(5) Surgery (Ophthalmology) - Univ. Santiago de Compostela, Santiago de Compostela*

**Purpose** The purpose of the present study was to develop mathematical relationships that allow obtaining equilibrium water content and refractive index of conventional and silicone hydrogel soft contact lenses from refractive index measures obtained with automated refractometry or equilibrium water content measures derived from manual refractometry, respectively.

**Methods** Twelve HEMA-based hydrogels of different hydration and the four currently existing siloxane based polymers currently available were assayed. Atago N-2E handheld refractometer and CLR 12-70 digital automated refractometer where used by two trained investigators. Polynomial models obtained from the sucrose curves of equilibrium water content against refractive index and vice-versa where used either considering the whole range of sucrose concentrations (16% to 100% equilibrium water content) or a range confined to the equilibrium equilibrium water content of current soft contact lenses (approximately 20% to 80% equilibrium water content).

**Results** Values of equilibrium water content measured with the Atago N-2E and those derived from the refractive index measurement with CLR 12-70 by the applications of sucrose-based models displayed a strong linear correlation (r2=0,978). The same values were obtained when the models are applied to obtain refractive index values from the Atago N-2E and compared with those (values) given by the CLR 12-70 (r2=0,978).

**Conclusion** Present results will have implications for future experimental and clinical research regarding normal hydration and dehydration experiments with modern hydrogel polymers, and particularly in the field of contact lenses.

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## Silicone-hydrogel contact lenses – microbial colonisation and hydrophobicity

*SANTOS L (1, 2), LIRA M (2), REAL OLIVEIRA MECD (2), OLIVEIRA R (1), AZEREDO J (1)*
*(1) Biological Engineering, Braga*
*(2) Center of Physics, Braga*

**Purpose** To study the relation between microbial colonisation and hydrophobicity of different types of silicone-hydrogel contact lenses (CL).

**Methods** The CL used in these work were Purevision™, Focus® Night & Day™, Acuvue®Advance™ with Hydraclear. It was also used a conventional disposable hydrogel Acuvue®. The experiments were performed after the CL removal of patients from both sexes with mean ages of 22.1 ± 4.2 years. Every patient used a conventional disposable hydrogel during 15 days and a silicone-hydrogel CL during 1 month, one in each eye, in a daily wear schedule. The extent of microbial colonisation was evaluated by the determination of the colonies formatting units (CFU). The hydrophobicity was determined through contact angle measurement using the advancing type technique with Millipore water.

**Results** Microbial colonisation was significantly higher (p<0.05) on CL wear by certain patients, in particular the one using the conventional hydrogel CL. The hydrophobicity of the conventional hydrogel CL increases after wear. The high degree of colonisation found on CL with high contact angles (p<0.05), suggests that hydrophobic interactions may have played an important role in microbial adhesion.

**Conclusion** There is a change in the surface properties of the CL with wear, influencing the extent of microbial colonisation.

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## Protein adsorption to silicone-hydrogel contact lenses – An in vivo study

*SANTOS L (1, 2), LIRA M (2), REAL OLIVEIRA MECD (2), OLIVEIRA R (1), AZEREDO J (1)*
*(1) Biological Engineering, Braga*
*(2) Center of Physics, Braga*

**Purpose** To analyse the protein adsorption to different types of silicone-hydrogel contact lenses (CL).

**Methods** The CL used in these work were Purevision™, Focus® Night & Day™, Acuvue®Advance™ with Hydraclear. It was also used conventional disposable hydrogel Acuvue® for comparison. The adsorbed proteins were recovered after the contact lenses removed from patients from both sexes with mean ages of 22.1 ± 4.2 years. Every patient used a conventional disposable hydrogel during 15 days and a silicone-hydrogel CL during 1 month, one in each eye, in a daily wear schedule. Proteins were analyzed by SDS-Page.

**Results** Conventional hydrogel and silicone-hydrogel CL used by the same patient exhibit different adsorbed proteins. This result suggests that the tear film proteins may establish different interactions with a conventional hydrogel and a silicone-hydrogel. It was also observed different proteins adsorbed on the several types of silicone-hydrogel CL used in this study. The CL with no surface treatment (Acuvue®Advance™ with Hydraclear) appears to adsorb a major amount of proteins compared with the one with surface treatment (Purevision™ and Focus® Night & Day™).

**Conclusion** The CL material influences, as well as the presence of a surface treatment, the type of tear film protein adsorbed.

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## PRK and LASEK in pediatric patients with high myopic anisometropia and amblyopia

*AUTRATA R, REHUREK J, VODIČKOVÁ K, PYROCHTOVA S*
*Department of Ophthalmology, Masaryk University Hospital,, Brno*

**Purpose** To evaluate visual and refractive outcomes of multizonal photorefractive keratectomy (PRK) and laser subepithelial keratomileusis (LASEK) for high myopic anisometropia and contact lens intolerance in 58 children.

**Methods** Group 1: Twenty six patients aged 7.1 to 15 years underwent PRK on the more myopic eye. Group 2: Thirty two patients aged 4 to 7 years with high myopic anisometropia and amblyopia had performed PRK (13 eyes) or LASEK (19 eyes) on the more myopic eye in general anesthesia, Excimer laser Nidek EC 5000 was used. Refractive surgical data reported in standard format to describe safety, efficacy, predictability, stability of the procedure. A long term binocular vision outcome was analyzed.. All patients completed 2 to 10 years follow-up. In children up to 8 years of age, surgery was followed by patching of the dominant eye.

**Results** Group 1: The mean preoperative spherical equivalent (SE) refraction was –8.72 D and the mean postop. SE –1.43D ($P<0.05$). The mean preop UCVA 0.038 increased to 0.41 ($P<0.05$) postop. The mean preop. BSCVA was 0.54 and changed to 0.68 postop. The safety index was 1.24. All the eyes had no line lost in BSCVA, 12 eyes had one line gained and 9 eyes had two lines gained Group 2: The mean preoperative SE refraction in Group A was –8.45 D decreased to D at 2-3 years postop. The mean preop. BSCVA 0.21 improved to 0.76 by 2-3 years postop. None of the eyes had grade 3 of haze. Postoperative binocular vision was saved or improved.

**Conclusion** PRK and LASEK, are effective and safe methods to reduce high myopic anisometropia in children aged 4 to 15 years and to improve amblyopia in children aged 4 to 7 years, when contact lens intolerance.

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## Phototherapeutic keratectomy in children with a long term follow up

*AUTRATA R, REHUREK J, VODIČKOVÁ K, PYROCHTOVA S, HELMANOVA I*
*Department of Ophthalmology, Masaryk University Hospital, Brno*

**Purpose** We evaluated efficacy, stability and safety of PTK for treatment of superficial corneal opacities, irregularities, epithelial instability. Authors present also the visual and refractive results after PTK combined with photorefractive keratectomy (PRK) in one procedure.

**Methods** The study includes the group of 56 children (62 eyes) aged 0.3 to 18 years (mean: 11,4 years). Postoperative follow–up time ranged from 1 to 10 years (mean: 6.2 years). The main goal of treatment were to improve visual acuity and to reduce or eliminate subjective ocular discomfort: pain, lacrimation and photophobia. Excimer laser NIDEK EC 5000 was used in PTK mode with 7.5mm transition zone. Thirty three eyes underwent only PTK, and 29 eyes were treated by PTK combined with PRK to reduce preop myopia (17 eyes) or hyperopia (12 eyes).

**Results** The BSCVA was improved in all children, and episodes of ocular pain or discomfort, lacrimation and photophobia diminished. The mean preoperative LogMAR of BSCVA 0.812 improved to mean value 0.217 at last visit 1 to 9 years postop. No eye lost a line of BSCVA. Twenty one children had 5 or more Snellen´s lines gain of the BSCVA, 17 children gained 4 lines, 11 children gained 3 lines and 7 children gained 2 lines postoperatively. At five cases were evaluated only 1 line gain of BSCVA. The mean preoperative SE of 17 myopic eyes decreased from –5.76 to –1.39 D and the mean SE of 12 hyperopic eyes changed from +4.47 D to +1.73 D by 2 to 5 years after the combined procedure.

**Conclusion** PTK in children is safe and effective treatment method of various surface corneal disorders. PTK may significantly improve visual acuity. Refractive error can be reduced by the combined procedure.

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## Inflow of Ocular Surface Fluid into the Anterior Chamber in Patients after Phacoemulsification through Sutureless Corneal Cataract Wounds

*BEHRENS A, STARK WJ, HERRETES S, PIROUZMANESH A, REYES JMG, MCDONNELL PJ*
*The Wilmer Ophthalmological Institute, The Johns Hopkins Hospital, Baltimore*

**Purpose** To report inflow of extraocular fluid after phacoemulsification using sutureless corneal incisions.

**Methods** 8 individuals (3 female), aged 58 to 91 years, showing minimal bleeding from the limbal capillary bed during phacoemulsification were selected for the study. Surgery was performed through a 2.8 mm limbal incision. External pressure simulating patient manipulation was applied before and after wound hydrosealing with an irrigation cannula. Inflow of blood-tinged tear fluid into the anterior chamber through the wound was monitored using digital video.

**Results** Inflow of extraocular fluid was observed in all eyes when the cannula was released, even after wound hydrosealing. Two patients showed spontaneous fluid inflow.

**Conclusion** Tested sutureless corneal incisions allow inflow of extraocular fluid into the anterior chamber after phacoemulsification. This may permit intraocular contamination leading to endophthalmitis.

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### Modified Microkeratome-Assisted Posterior Lamellar Keratoplasty Using A Tissue Adhesive

*BEHRENS A (1), PIROUZMANESH A (1), HERRETES S (1), REYES JMG (1), SUWAN-APICHON O (1), CHUCK RS (1), WANG DA (2, 3), ELISSEEFF JH (2, 3), STARK WJ (1)*
*(1) The Wilmer Ophthalmological Institute, Johns Hopkins University, Baltimore*
*(2) Departments of Cell Biology, Johns Hopkins University, Baltimore*
*(3) Biomedical Engineering, Johns Hopkins University, Baltimore*

**Purpose** To compare graft stability and astigmatic change using suture vs. tissue adhesive in an experimental model of microkeratome-assisted posterior lamellar keratoplasty (PLK).

**Methods** A 300-µm thick flap-keratectomy after incomplete pass was performed in human donor corneoscleral rims using an artificial anterior chamber and a manual microkeratome. The flap hinge at the left central opening border, providing a wide hinge to add stability. After flap reflection, a 6.25 mm trephination was performed obtaining a disc of posterior stroma, Descemet's membrane, and endothelium. The disc was positioned in a sutureless fashion, and the flap secured with either 5 interrupted sutures, or a chondroitin-sulfate-aldehyde based adhesive. Increasing intrachamber pressures were created detecting graft stability. Videokeratographic data was recorded evaluating astigmatic change.

**Results** The mean astigmatic change was 3.08 D (0.84) in the sutured group, and 1.13 D (0.55) in the glued group (p=0.008). Mean resisted pressures were 95.68 (27.38) mmHg and 82.45 (18.40) mmHg in the sutured and glued groups, respectively (p=0.97).

**Conclusion** This modified technique of microkeratome-assisted PLK showed excellent graft stability in both groups. Flaps sealed with the novel tissue adhesive had reduced astigmatic changes in our experimental model.

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### Keratocyte apoptosis in keratoconus: A function of TIMP-3 and TIMP-1 synthesis?

*SMITH VA (1), MATTHEWS FJ (2)*
*(1) Ophthalmology Unit, Bristol*
*(2) Department of Virology, Bristol*

**Purpose** The thinning of keratoconic corneas could result from keratocyte apoptosis or be induced or perpetuated by the activation of matrix degrading enzymes, particularly those of the MMP family. The MMP inhibitors TIMP-3 and TIMP-1 may exhibit apoptotic and anti-apoptotic properties respectively. Because of their potential to influence keratoconus progression, the effects of these proteins on keratocyte viability were investigated.

**Methods** Keratocyte cultures were infected with RAdTIMP-3 and RAdTIMP-1. The expressed proteins were quantified by ELISA. Apoptotic cells were detected by TUNEL and caspase-3 activity. The anti-apoptotic effects of TIMP-1 were investigated by RAdTIMP-1 and RAdTIMP-3 coinfection and by adding TIMP-1 protein to keratocyte cultures prior to infection with RAdTIMP-3. Immunohistochemistry was used to localise and determine relative numbers of apoptotic and TIMP producing keratocytes in normal and keratoconic corneal sections.

**Results** TIMP-3 over-expression induced keratocyte apoptosis. Upregulated TIMP-1 production or the addition of exogenous TIMP-1 protein prevented keratocyte overgrowth, changed keratocyte morphology and reduced the extent of TIMP-3 induced apoptosis. In vivo significantly more apoptotic cells were identified in the anterior stroma of keratoconic corneas than normal corneas and the majority of theTIMP-3 and TIMP-1 producing keratocytes were also located in this region.

**Conclusion** TIMP-3 over-expression induced apoptosis in keratocytes cultured from normal corneas. TIMP-1 protected these cells against TIMP-3 induced apoptosis. Localised relative concentrations of TIMP-3 / TIMP-1 may thus determine whether a keratocyte becomes apoptotic or remains viable. This may be relevant to the keratoconic condition.

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### Agreement between two non-contact specular microscopes: TOPCON SP2000 versus RHINETEC

*THURET G (1, 2), NGUYEN F (1), ZHAO M (2), DEB N (2), GAVET Y (3, 2), GAIN P (1, 2)*
*(1) Ophthalmology dept, University hospital, Saint-Etienne*
*(2) Lab 'Cell survival and adhesion in cancer and graft' EA3063, faculty of medicine, Saint-Etienne*
*(3) Ecole Nationale Superieure des Mines, Saint-Etienne*

**Purpose** The new Rhinetec non-contact specular microscope (Germany) determines endothelial cell density (ECD) by a cell center method, either automatically or after manual touch-up. Aim: to determine its agreement with the widely widespread Topcon SP-2000 (Tokyo, Japan). This later is based on cell boundaries detection and requires extensive manual corrections of cell contours to be valid

**Methods** Successive assessments of the central ECD of 229 eyes were performed by a single ophthalmologist (FN). The Bland Altman method was used to determine the agreement between Topcon with manual corrections and Rhinetec with the automatic method and with manual corrections

**Results** Among the 229 corneas, 174 where form healthy eyes, 41 post graft, 7 post cataract surgery and 7 other. Mean age was 49 (11 to 93), median 45. ECD (Topcon touched-up) ranged from 476 to 3263 cells/mm². Numbers of counted cells were similar: 137+/-40 (Topcon) vs 142+/-42 (Rhinetec) p=0.067. Agreement between automatic Rhinetec and touched-up Topcon was poor, with a mean difference of 190 cells/mm² (Rhinetec>Topcon) with limits between -636 and +912 cells/mm². This automatic method overestimated low ECD (<1500) and underestimated high ECD (>2500). Agreement between both manual methods was high with a mean difference of 12 cells/mm² (Rhinetec>Topcon) and limits set between -402 and +427 cells/mm². The tendency to overestimated low cell density and underestimate high slightly persisted

**Conclusion** Full automatic methods must definitely be rejected. Rhinetec shows a good agreement with Topcon SP2000 when extensive manual corrections are performed. Nevertheless, regarding the two bounds of agreements on this sample, one must accept a possible consistent individual variation

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### Osteo-odonto Keratoprosthesis: A 40-Year Review

*MICHAEL R (1), SRIPATTANAWAT R (1), ALVAREZ A (1), TEMPRANO J (2), DE LA PAZ MF (2), CHAROENROOK V (2), ALVAREZ DE TOLEDO J (2), BARRAQUER RI (2, 1)*
*(1) Institut Universitari Barraquer, Barcelona*
*(2) Centro de Oftalmologia Barraquer, Barcelona*

**Purpose** To study short and long term functional results after Osteo-Odonto Keratoprosthesis (OOKP).

**Methods** We reviewed the charts of 287 patients who underwent osteo-odonto keratoprosthesis at the Barraquer Centre of Ophthalmology from January 1964 to May 2005. A total of 337 cases, 100 of which were bilateral were reviewed; 219 were male and 116 were female. All surgeries were done by a single surgeon using Strampelli´s technique with a variation using tibia (Tempranó's technique) if patient was edentulous. Functional success was defined as visual acuity > 0.05 (legal blindness as defined by the WHO). Survival rates were calculated using life tables and the Kaplan-Meier estimator.

**Results** Mean follow-up time was 74 months (range: 1-585 months). Mean age of patients was 42 years (range: 4 – 86 years). Prior to intervention all patients were legally blind (VA < 0.05) and at final follow-up 153 patients had visual acuity better than 0.05. Overall survival rate was 86% at 1 month, 71% at 1 year, 50% in 5 years, 38% in 10 years and 18% in 25 years. Complications encountered were cataract (61%), vitritis (36%), expulsion (35%), glaucoma (19%), aseptic necrosis (19%), retinal detachment (16%). Survival rates for chemical burn (135 cases), Stevens-Johnson Syndrome (39 cases), Ocular cicatricial pemphigoid (16 cases) and other aetiologies are presented.

**Conclusion** A 50% functional success rate after 5 years is quite satisfactory for these end-stage ocular-surface diseases.

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## The assessment of corneal endothelium before and after cornea storage in tissue culture conditions

*JIRSOVA K, TOMSLI S, ANDRLE J, FILIPEC M*
*Laboratory and Ocular Tissue Bank, Dept of Ophthalmology, General Teaching Hospital and Charles University, Prague*

**Purpose** The aim of this study was to compare the endothelial cell density, the mean number of live/dead cells per mm2, the coefficient of variation and the degree of hexagonality before and after preservation of human corneas under tissue culture (TC) conditions.

**Methods** Photographs of the central part of 120 corneas, taken before and after storage in tissue culture at 31°C for periods ranging from one to four weeks, were analysed. The endothelial cell density (ECD) and the number of dead cells per mm2 were calculated from phase contrast and bright field photographs, respectively. All photographs were encoded and the assessment was performed separately by two experienced observers using a Lucia computer analysis system. The data from all storage periods were combined and processed by the same system to determine the following parameters: endothelial cell density, dead endothelial cell density (D-ECD), live endothelial cell density (L-ECD), coefficient of variation of cell area, (CV) and percentage of hexagonal cells, (6A).

**Results** The mean ECD before cultivation was 2773 cells/mm2, L-ECD was 2728 cells/mm2 and D-ECD was 45 cells/mm2. During the cultivation, the mean ECD value decreased to 95%, L-ECD to 96% and D-ECD to 16% of original values. The degree of hexagonality decreased from 0.54 before, to 0.51 after storage. The decreases of all these parameters were significant, while polymeghatism was the only value that was not significantly changed (0.20 before and after TC).

**Conclusion** The significant decrease in the number of dead cells during TC storage up to 5 weeks was observed. This leads to the conclusion that the TC method is especially indicated for the preservation of corneas with higher numbers of dead cells.

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## The presence of HLA-DR positive cells in human corneas stored under hypothermic or tissue culture conditions

*AL FAKIH A (1), JIRSOVA K (1), FILIPEC M (2)*
*(1) Laboratory and Ocular Tissue Bank,Deparment of ophthalmology, Prague*
*(2) Ophthalmology, Prague*

**Purpose** To compare the number of HLA-DR positive cells in fresh corneo-scleral discs and in discs stored under hypothermic or tissue culture (TC) conditions.

**Methods** Corneo-scleral discs excluded from the transplant program were used. Four unpreserved corneo-scleral discs, 16 stored in hypothermic (Optisol-GS,4°C), and 20 stored in TC (31°C) for 3-5, 7-9, 12-14, and 20-24 days, and 28 days (for TC only) were used. All discs were dissected into four concentric zones: central, pericentral, peripheral cornea and limbo–scleral zone. The tissue was snap-frozen in liquid nitrogen and stored at -70°C until processed. 7 µm thick sections were fixed and streptavidin-biotin complex/alkaline phosphatase indirect imunohistochemistry using anti HLA-DR (Immunotech, France) was performed. HLA-DR positive cells were counted using a Lucia analysis system.

**Results** The number of HLA-DR positive cells detected in the epithelium was 3 cells/mm² in central and pericentral cornea, 7 cells/mm² in periphery, 20 cells/mm² in limbal, and 33 cells/mm² in conjunctival epithelium. A comparable decrease in the number of HLA-DR positive cells were detected in epithelium of corneo-scleral discs stored in hypothermic and TC conditions for 3-5 days. Complete absence of HLA-DR positive cells was observed after 12-14 days in hypothermic and 7-9 days in TC conditions. The disappearance of HLA-DR positive cells in limbus and conjunctiva occurred after 20-24 days and more than 28 in hypotermic or TC storage, respectively.

**Conclusion** The decrease of HLA-DR positive cells is more pronounced in TC compared to hypothermic storage. The absence of HLA-DR cells in corneas stored in TC longer than 7 days could be favorable in preventing corneal graft rejection.

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## Presence of IgE Antibody and Kinin Components in Atopic and Non-Atopic Contact Lens Wearing Patients

*MANN AM, TIGHE BJ*
*Biomaterials Research Unit, Birmingham*

**Purpose** The anterior ocular surface is a common target in the allergic response, however very little detail is known about the mechanisms involved or the added complications that ensue during, or as a consequence, of contact lens wear. The present work incorporates a variety of studies in which IgE and kinin activity was determined in contact lens wear and also provides an insight into their individual detection profiles and synergistic interactions. Additionally as a second objective, complementary studies are introduced which analyse the individual tear protein profile of patients using the very sensitive and reproducible results acquired on the 2100 Agilent Bioanalyser.

**Methods** The extracted lens deposits and tear 'fingerprints' were analysed by immunodiffusion assay and protein sizing lab-on-a-chip technology respectively. A variety of contact lenses over a range of modalities and materials were tested in both non-atopic subjects and those with existing allergies.

**Results** There was a progressive and significance increase in the percentage of subjects presenting with kinin and IgE moieties in their lens deposits between months one and twelve months. IgA levels were distinctly different between the control, normal subjects and those identified with allergies; the levels of tear IgA for the normal subjects were in the region of 425[FU] whereas the atopic subjects presented levels of approximately 125[FU]. Changes in IgA levels over time in contact lens wearers was determined to be patient and symptom dependent.

**Conclusion** The assessment and analysis of immunological changes in the tear film and during contact lens wear is important in discerning ocular complications.

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## Expression Of Focal Adhesion Protein PINCH In Alkali Injured Corneas And Role Of PMNs

*BOURGHARDT PEEBO B, GAN L, FAGERHOLM PPP*
*Dept. of Ophthalmology, University Hospital, Linköping*

**Purpose** Particularly interesting new cystein-histidin-rich protein (PINCH) functions as an adapter protein for signal transduction in the integrin and growth factor pathway. The purpose is to study the expression of PINCH in normal versus alkali wounded corneas and the influence of PMNs.

**Methods** The right eye of 14 New Zealand White rabbits were injured with a central penetrating 5 mm in diameter alkali injury in general anaestesia. Seven of the rabbits were injected intravenously with fucoidin every two hours for 36 hours. All rabbits were sacrificed after 36 hours and the corneas were excised with a scleral rim and fixed in 4% formaldehyde and embedded in paraffine. 4 µm thick sections were then immunohistochemichally stained using a polyclonal PINCH antibody.

**Results** PINCH was not expressed in the unwounded normal cornea or in the vascular endothelium of the limbal vessels. In the injured cornea the cells repopulating the wound after 36 hours, epithelium, stroma and endothelium all expressed PINCH in the frontline. In the limbal area the expression of PINCH was extensive in stromal, vascular endothelial and inflammatory cells. In the corneas from fucoidin injected rabbits, the expression of PINCH was positive in the repopulating cells although weaker. In the limbal area the expression of PINCH was strongly reduced but still somewhat more discernible in the stromal cells compared to unwounded corneas.

**Conclusion** PINCH is a marker for several types of cell activities and is, like in tumor growth, expressed during wound healing. PINCH is also expressed in the limbal vascular endothelium when there is stimulation for neovascularization. The absence of PMNs prevents the expression of PINCH in the vascular endothelium.

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## Corneal wound healing after intrastromal femtosecond laser keratectomy

*MELTENDORF C (1), BURBACH GJ (2), BÜHREN J (1), OHRLOFF C (1), DELLER T (2)*
*(1) Department of Ophthalmology, Johann Wolfgang Goethe-University, Frankfurt am Main*
*(2) Institute of Clinical Neuroanatomy, Johann Wolfgang Goethe-University, Frankfurt am Main*

**Purpose** The purpose of this project was to examine the corneal repair response after intrastromal femtosecond keratectomy which is characterized by the absence of epithelial damage after surgery.

**Methods** Twelve rabbits underwent monocular intrastromal keratectomy at a corneal depth of 200 µm using a femtosecond-laser. Follow-up examinations were performed 1, 3, 7, and 28 days after surgery. Corneas were evaluated using slit lamp and in vivo confocal microscopy. Immunofluorescence microscopy was used to localize fibronectin and tenascin C as markers of early stromal wound healing. Nuclear DNA fragmentation was detected with the TUNEL assay. Anti-α-smooth muscle actin was used to determine if myofibroblasts were present.

**Results** On slit-lamp examination, the corneas were transparent 1 day after surgery. Keratectomy was detectable as a narrow opaque band that was still visible after 28 days. After 1 day, TUNEL-staining revealed a broad zone of TUNEL-positive keratocytes. Immunofluorescence studies disclosed the deposition of fibronectin and tenascin C in the same area already 1 day after surgery. In vivo confocal microscopy showed a regular pattern of highly reflective laser spots and an irregular network of brightly reflecting keratocytes within the keratectomy zone after 1 day. After 28 days, this zone showed unstructured acellular areas with increased reflectivity. No α-smooth muscle actin was detected at any time point.

**Conclusion** Keratocyte apoptosis occurs at the zone of intrastromal keratectomy despite the absence of a concomitant epithelial debridement. The lack of myofibroblast formation indicates that the integrity of the basement membrane might be a crucial factor in limiting the fibrotic repair response.

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## NGF treatment and corneal wound healing

*BLANCO-MEZQUITA JT (1), MERAYO-LLOVES J (1), MARTÍNEZ-GARCÍA C (2), MAR-SARDAÑA S (3), MARTÍNEZ-OSORIO H (1), TORRES R (1), GONZÁLEZ-PARRA M (1), BONINI S (4), LAMBIASE A (4)*
*(1) IOBA, Valladolid*
*(2) CELL BIOLOGY, Valladolid*
*(3) OPTICS AND APPLIED PHYSICS, Valladolid*
*(4) LABORATORY OF OPHTHALMOLOGY, Rome*

**Purpose** The purpose of this research is to evaluate the effectiveness of topical administration of murine Nerve Growth Factor in the wound healing process after refractive surgery

**Methods** Iber Braun hens underwent PRK were divided into different groups treated with topical administration of 0.2% murine Nerve Growth Factor (NGF) group A, Balanced Salt Solution (BSS) group B and group C received no treatment. Fluorescein staining, clinical follow up of haze, pachymetry, and both transmitance and scattering measurements were taken. Eyes were exenterated at sequential time points and fixed in 10% buffered formalin. Sections were stained with H-E. TUNEL technique was used for apoptosis detection. Antibodies anti-BrdU were used for cell proliferation. Antibodies anti-□-SMA were used for myofibroblast differentiation. TrkA receptors and cytochrome c were detected by immunohistochemestry.

**Results** There was not difference in the recovery of the epithelium integrity between groups. Differences in earlier apoptosis , cell proliferation, cell differentiation and cytocrome c location were found between groups. Statistically significant differences between the appearance and disappearance of the haze, pachymetry, transmitance and scattering were found.TrkA receptors was found in the epithelium, endothelium ans keratocytes in all the corneas

**Conclusion** In normal corneas, NGF has not effect in the recovery of epithelium integrity Stromal apoptosis, proliferation and myofibroblast differentiation were modulated by NGFTrkA receptors are present in the cornea of hens which remarks that this is a very good animal model to study corneal wound healing after refractive surgery

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## The Nature and Fate of Tear Lipids in Contact Lens Wear

*MANN AM, CAMPBELL D, GRIFFITH G, TIGHE B*
*Biomaterials Research Unit, Birmingham*

**Purpose** The role & structure of the layers of the tear film is a subject of ongoing discussion in ophthalmology and the increased use of silicone hydrogels has produced renewed interest in the interaction of lipids with contact lenses. In particular the polar lipids & their ability to act as an aqueous-lipid interface are of importance. Oxidation of lipids leads to their degradation and dysfunction. This poster shows techniques that are being developed to probe the role of polar tear lipids & to study the interaction of the tear film lipid layer with (silicone hydrogel) contact lenses

**Methods** Chromatographic, fluorescence & mass spectrometric are used for compositional analysis of the lipid layer of tear film. Spinning drop tensiometry is used to study the interfacial chemistry of the individual lipids and the tear film. Tear lipid degradation products are analysed using MDA and FOX analysis. The depth of lipid penetration into lenses is investigated using novel techniques (PEEMS & PEEFS). The effects of introducing lenses into the tear film will also be investigated using these techniques. Lenses & tear samples are obtained from a variety of clinically managed programmes

**Results** Irrespective of composition or surface coating technique lipid accumulation occurs rapidly on all commercially available silicone hydrogel lenses. Spinning drop tensiometer studies show that the polar phospholipids are essential to enable the rapid spreading of the lipid layer over the aqueous.

**Conclusion** While broad similarities exist in lipid composition between subjects the analyses of the phospholipids and lipid oxidation products are of interest to establish their correlation with patient-related wear problems

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## Visual recovery following PRK using corneal epithelium brushing and alcoholic dehydration : a confocal microscopy study

*PICCIRILLO V, DI NUZZO A, RAO V*
*Ospedale Civile di Maddaloni ( ASL CE / 1 ), Maddaloni*

**Purpose** To study visual recovery and confocal microscopy findings three months after PRK using mechanical (brushing) and chemical (alcoholic) corneal epithelium removal .

**Methods** 120 corneas of 60 patients (30 males,30 females,average:36.4 y. o) were examined by slit-scanning confocal microscope (CONFOSCAN 2) 3 months after correction of simple myopia (average - 5.50 D. ) using PRK technique ( Laser Schwind ) . Male and female patients were at random divided into two groups :group 1,who received corneal epithelium brushing, group 2,who received epithelium dehydration.Patients were matched for age,sex and ethnicity.Visual recovery (Snellen chart)and Confoscan findings were related in the short term follow up (3 months) .

**Results** 3 months after PRK mean visual acuity was not different between the groups. Confocal microscopy did not reveal morphological differences within the corneal epithelium and anterior part of stroma between the groups :irregular pattern of elongated keratocytic nuclei were found in 50 % of both groups patients.The only difference was the rapidity of corneal riepithelization after PRK as evaluated by slit lamp:a complete corneal riepithelization was observed in the forth day in 90% of group 1 patients,in the fifth day in 60% of group 2 patients .

**Conclusion** Mechanical and chemical corneal epithelium removal does not influence visual recovery after PRK .Confoscan examination ( CONFOSCAN 2 ) does not reveal morphological differences between the techniques within the corneal epithelium and anterior part of stroma . The rapidity of complete corneal riepithelization is higher in patients treated with corneal brushing as revealed by slit lamp examination .

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## Influence of multipurpose solutions on the protein composition of the tear film

*GRUS FH, BOZKURT N, PFEIFFER N*
*Dept. of Ophthalmology, University of Mainz, Mainz*

**Purpose** To analyze the tear protein profiles of non-contact lens wearers, and of contact lens wearers using different multipurpose solutions (MPS) for cleaning and storage.

**Methods** Wearers of soft contact lenses were recruited and allocated to use either Optifree Express MPS or Complete MPS for 4 weeks (n = 20 in each group). Tears were collected and analyzed before starting use of solutions, and at 1, 2, and 4 weeks after starting use. Tears were also collected and analyzed from 20 control patients (non-contact lens wearers), who were not exposed to either MPS. Multivariate statistical analysis of protein profiles was used to determine the normality of tear protein composition. Specific protein biomarkers were found by means of ProteinChips (SELDI-TOF) with subsequent multivariate statistics and artificial neural networks, and identified using tandem mass spectrometry (LC-MSMS).

**Results** Before starting use of solutions, tear protein composition in all contact lens wearers deviated from tear composition in normal controls (non-contact lens wearers). After 4 weeks using the different care regimens, tear protein composition of patients using OptiFree Express MPS was further deviated from normal. In contrast, tear protein composition of patients using Complete MPS returned towards normal. In fact, the tear composition of over 50% of Complete MPS users was classified as "normal" rather than "contact lens wearer" at 4 weeks. Using MPS, a decrease of inflammatory markers and an increase of potentially protective markers could be clearly demonstrated.

**Conclusion** Contact lens wear alters tear protein profiles in a complex manner. The use of MPA solutions such as Complete returns the tear profile towards normal.

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## Frictional and lubricity changes in contact lenses during wear

*MAHOMED A, NASSO M, TIGHE BJ*
*Biomaterials Research Group, Aston University, Birmingham*

**Purpose** Friction and lubricity are important aspects of the interaction between contact lenses and the anterior eye and the in vivo behaviour of ophthalmic solutions. Although instrumentation is available for the measurement of artificial joint components, there are as yet no established in vitro techniques that reflect the sliding motion between the eyelid and the anterior surface of the lens during blinking. We have investigated technique development in this area in order to measure the coefficient of friction (μ) of contact lenses before and after wear.

**Methods** A high sensitivity tribometer has been identified and adapted for the study of contact lenses. The lens is placed on a convex mould which slides against a moving substrate (which can be varied) in the optional presence of an appropriate lubricating solution (tears or artificial tear solution). The resistance to motion is expressed in terms of μ.

**Results** Using this instrument μ values down to 0.001 can be measured reproducibly. Lenses from a series of clinically managed wearer trials have been studied. The technique shows clear material-dependant differences in μ values of unworn lenses (0.005–0.1). More interestingly are changes in the frictional behaviour of lenses as a consequence of wear (0.05-0.2).

**Conclusion** Three distinct contributions can be discerned. The first is due to progressive front surface dehydration during wear, observed only if lenses are examined directly on removal from the eye. The second is due to deposition and subsequent degradation of tear components, principally lipid and protein. The third is due to changes in the surface chemistry of the lens material, typified by, and most marked in, the loss of polyvinyl alcohol during wear from Focus® Dailies® lenses.

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## Novel Ophthalmic Biomaterials

*MAHOMED A, HENLEY C, TIGHE BJ*
*Biomaterials Research Unit, Aston University, Birmingham*

**Purpose** The synthesis of new hydrogels with suitable properties to enable them to be used as biomimetic synthetic cornea, corneal inlays & onlays, & "third generation" contact lens materials.

**Methods** The human cornea provides precise targets in terms of mechanical & surface properties. Due to the cornea having a complex & composite structure it is not possible to mimic its properties with homogenous hydrogels. The approach used here is to synthesise Semi Interpenetrating Polymer Networks, which mimic to some extent the Interpenetrating Polymer Network of natural structures such as cornea & intervertebral disc. To achieve this it has been necessary to extend the range of monomers conventionally used in ophthalmic biomaterials in order to obtain a wider range of solvation & partitioning properties of the constituent monomers.

**Results** A group of N-vinylamides used singly or in combination has allowed the target structure to be achieved. These monomers have also enabled the synthesis of a range of novel copolymers, which are particularly useful in the synthesis of contact lens material that combines flexibility with high oxygen permeability. A hydrophilicity series of these amides has been determined by measuring the Equilibrium Water Contents of copolymers consisting of 70%(w/w) 2-hydroxyethylmethacrylate and 30%(w/w) N-vinylamide. The following series was obtained: N-vinylacetamide (63%) > N-methyl-N-vinylacetamide (NMNVA) (53%) > N-vinylpyrolidone (48%). In addition NMNVA has been found to have excellent solvation properties, is compatible with a variety of conventional monomers, & has low cytotoxicity.

**Conclusion** N-vinylamides are a group of monomers that can potentially revolutionise ophthalmic biomaterials in terms of their excellent solvation, partitioning & compatibility properties.

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## Silicone Hydrogels: Trends in Products and Properties

*FRANKLIN V, ROSS G, NASSO M, LYDON F, TIGHE B*
*Biomaterials Research Unit, Birmingham*

**Purpose** Although silicone hydrogels resemble conventional hydrogels because of the water that they contain, the substantial presence of relatively hydrophobic silicone components leads to many differences in behaviour from that of simple mid to high water content hydrogel lenses. In the six years since their launch, clinical experience has revealed a combination of characteristic benefits and complications (such as mucin balls and SEALS).

**Methods** This poster compares the dynamic mechanical properties, dynamic wettability, and frictional properties of galyficon A, lotrafilcon-A and balafilcon-A (together with such other materials as become available for characterisation) in comparison to two reference points. The first of these is typical conventional hydrogel behaviour in the mid-water content range. The second is the human cornea.

**Results** Taken together these properties provide a basis for interpreting the clinical behaviour of silicone hydrogels in comparison with conventional soft lens materials.

**Conclusion** The relative behaviour of materials will be summarised on the poster and the fact that current silicone hydrogels are significantly different fromconventional hydrogels and also from each other highlights the need for standardised relevant in-vitro methods of assessment for these materials

## ▪ 241 / 4464
## Immunomodulatory Role of VIP in a Murine Model of P. aeruginosa-Induced Keratitis

*SZLITER EA, LIGHVANI S, HAZLETT LD*
*Anatomy & Cell Biology, Detroit*

**Purpose** In P. aeruginosa ocular infection, dominant Th1-responsive mouse strains are susceptible (cornea perforates), while Th2-responsive mouse strains are resistant (cornea heals). Neuropeptides are endogenous neuroendocrine factors recently correlated with neuroimmune interaction and function, but little is known of their role in mediating/regulating ocular infectious disease. Previous studies have shown an increased level of vasoactive intestinal peptide (VIP) expression, a neuropeptide associated with potent anti-inflammatory activities, in BALB/c (Th2-responder) vs. C57BL/6 (B6) (Th1-responder) mice. Therefore, this study further examined the role of VIP in modulating the host immune response to P. aeruginosa-induced keratitis.

**Methods** B6 mice were injected i.p. with recombinant (r) VIP daily from -1 through 7 days p.i. Control mice were similarly injected with PBS. Clinical scores, slit-lamp, bacterial plate counts, myeloperoxidase to quantitate PMN, real-time RT-PCR and ELISA were used to assess the effects of rVIP treatment in modulating disease pathogenesis.

**Results** Injection of B6 mice with rVIP prevented corneal perforation, normally seen in the susceptible animal by 5-7 days p.i. Real-time RT-PCR analysis showed significantly decreased levels for both IFN-g and IL-1b mRNA at 7 days p.i. in rVIP corneas. Bacterial load was comparable for rVIP vs. control mice, while fewer PMN were present with time in rVIP-treated mice.

**Conclusion** These data provide evidence that VIP is a regulatory molecule of ocular inflammation. rVIP treatment counterbalanced the production of pro-inflammatory cytokines (IFN-g and IL-1b) and down-regulated PMN infiltration sufficiently to ameliorate the host-induced component of bacterial-induced inflammatory disease.

## ▪ 242 / 4465
## Corneal toxicity study of Perfluorohexyloctane in rabbit eyes

*DIAFAS SG (1), LAKE S (1), KARAMPATAKIS VE (2), KARDASOPOULOS AV (1), STANGOS NT (1), MANTHOS AK (3)*
*(1) Ophthalmology, Aristotle University of Thessaloniki, Thessaloniki*
*(2) Laboratory of Experimental Ophthalmology, Aristotle University of Thessaloniki, Thessaloniki*
*(3) Laboratory of Histology-Embryology, Aristotle University of Thessaloniki, Thessaloniki*

**Purpose** To evaluate the effects of perfluorohexyloctane (F6H8), a semi-fluorinated fluorocarbon of low specific gravity, on the rabbit cornea.

**Methods** 6 pigmented rabbits underwent injection of 0.1 ml of F6H8 in the anterior chamber of the right eye and BSS in the left eye. The rabbits were sacrificed after 24 days, the eyes were then enucleated and 1 specimen from the upper and 3 from the lower cornea of each eye were taken and processed for transmission electron microscopy (TEM). The specimens of the inferior cornea in contact with the F6H8 were then compared with the specimens of the superior cornea.

**Results** Postoperatively the inflammation in eyes receiving F6H8 was more severe than in the controls. In the anterior chamber, the formation of small bubbles, "fish egging" phenomenon, was observed. A ridge was observed in 2 eyes, as a landmark limiting the area of contact with the F6H8, with conjunctival congestion and cornea cloudiness, without corneal neovascularization.On TEM all the control eyes (BSS) were free of pathological changes. Morphological lesions were found in the area in contact with the F6H8 in the inferior cornea. Vacuoles of F6H8 were present within the endothelium but not in the corneal stroma. Widening of the endothelial microvilli was present and also flattening of the endothelium cells and their nuclei. Formation of a multi layer retrocorneal fibrous membrane was observed in conjunction to the endothelial cells in 2 eyes.

**Conclusion** Our data indicate that F6H8 induces corneal toxicity in rabbits when present in eyes for 24 days. We therefore recommend caution in patients without barrier to prevent the fluid migrating from the vitreous cavity into the anterior chamber.

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## Corneal Crystallin Expression in Human Repair Phenotype Keratocytes

*MCDERMOTT AM, PEI Y, SHERRY DM*
*College of Optometry, Houston*

**Purpose** To compare expression of the putative corneal crystallins aldehyde dehydrogenase (ALDH) and transketolase (TKT) in human corneal fibroblasts, myofibroblasts and penetrating keratoplasty (PKP) specimens.

**Methods** Isolated human keratocytes were cultured with serum or serum and TGF-beta to obtain corneal fibroblasts and myofibroblasts respectively. RT-PCR, immunoblotting and labeling were used to detect expression of ALDH isoforms and TKT. ALDH3A1 activity was quantitated by a colorimetric assay. PKP tissue sections were immunolabeled for ALDH3A1, Thy-1 (a repair phenotype marker) and alpha-smooth muscle actin (alpha-SMA, a myofibroblast marker).

**Results** Keratocytes expressed ALDH 1 and 3A1 mRNA (n=3), and 1 of 3 samples expressed ALDH 2 and TKT. Corneal fibroblasts expressed ALDH 1 and 3A1 mRNA (n=3) and there was a 26% decrease in ALDH3A1 mRNA expression in fibroblasts compared to keratocytes (n=2). ALDH3A1 protein was readily detectable in keratocytes but was not present in corneal fibroblasts or myofibroblasts (n=3). ALDH3A1 enzyme activity was markedly lower in fibroblasts than keratocytes (14 +/- 12 vs 135.6+/-73 U/mg protein, n=3). Repair phenotype keratocytes (Thy-1 or alpha-SMA positive) were present in 2 of 6 PKP specimens. These cells did not stain for ALDH3A1 whereas all normal keratocytes (Thy-1 or alpha-SMA negative) were ALDH3A1 positive.

**Conclusion** These results confirm in human cornea, the results of previous animal studies showing decreased ALDH3A1 expression in repair phenotype keratocytes. The significance of the decrease remains to be determined but may contribute to enhanced reflectivity of the repair phenotypes and hence stromal haze.

## ▪ 244 / 4467
## Numerical simulation of corneal transport processes

*TIGHE B, LI LY*
*School of Engineering and Applied Science, Birmingham*

**Purpose** Modeling of corneal swelling and its environmental dependence is critical to an understanding of corneal function, particularly when important physiological parameters are refractory to experimental investigation. For the cornea, endothelial membrane transport parameters are difficult to access directly, thus development of an effective stromal transport model will enable isolation of endothelial membrane transport properties.

**Methods** Transport equations for each ionic species and ionic solution within the corneal stroma are derived based on transport processes developed for electrolytic solutions, whereas the transport across epithelial and endothelial membranes is modeled using phenomenological equations derived from thermodynamics of irreversible processes. Time-dependent effects are then derived.

**Results** For given initial ionic concentrations and stromal hydration levels together with boundary conditions (ionic concentrations and hydrostatic pressures in tears and aqueous humour) partial differential equations are solved using finite element methods, to obtain the hydration distribution, and thus the thickness variation. . The greatest change in thickness is found for the combination of sodium and bicarbonate ions whilst least change is found with the combination of potassium and chloride.

**Conclusion** A computational model of corneal hydration has been proposed for simulating the response of corneal thickness to perfusion with hypotonic or hypertonic solutions.This has been used to demonstrate the influence of flow across the epithelium and endothelium on the stromal thickness response and the role of the ionic active pumps in epithelial and endothelial layers.

## ▪ 245 / 4468
## Cellular activity in human regrafts 1 – 30 years primary surgery

*GAN L (1), FAGERHOLM PPP (1), STENEVI U (2)*
*(1) Ophthalmology, Linköping*
*(2) Ophthalmolofy, Göteborg*

**Purpose** A previous study by the group have revealed that the cells in 60 studied human corneal grafts are to a large extent replaced also in the stroma and in the endothelium. The time to reoperation was between 1 and 30 years. The interest of this study was to see cellular activities in these corneas at the time of retransplantation.

**Methods** We used immunohistochemichal markers for proliferative cell nuclear antigen (PCNA), Caspase 3, S 100 A4, PINCH and VEGFr-3 to study the expression in formaldehyde fixed, paraffin embedded corneal buttons retrieveded at reoperation1-30 years after the primary transplantation. Eye bank corneas and 3 corneal buttons retrieved at primary corneal grafts surved as reference material.

**Results** PCNA was expressed in all cell layers in the majority of the 19 specimens. 46 cells/ section were positive in the epithelium and 33 cells/ section in the stroma on average. In the endothelium, 11 % of the endothelial cells expressed PCNA. These figures were slightly higher in the 5 reference corneas. Caspas 3 expression was found in half of the specimen in all cell layers but very few in numbers. There was a discrepancy in the expression of S 100 A4 and PINCH in that S100 A4 expressed extensively and PINCH rather restricted both in all cell types.VEGFR-3 was expressed in all the rejected grafts as well as other grafts which regrafted by others reasons. The cell types of VEGFR-3 positivity found are case related.

**Conclusion** The cell proliferative activity can be seen in regrafts in all cell types. The apoptotic activity exists but is scarce. Markers for mobility, S 100A4 and PINCH, expresses differently in different cases. VEGFr-3 is involved in human regrafts. The role of VEGFr-3 in corneal immune-rejection need further studies.

## ▪ 246 / 4469
## Evaluation of Megacell MEM as a corneal storage medium

*SMITH VA*
*Ophthalmology Unit, Bristol*

**Purpose** Bristol CTS Eye Bank corneas are currently held in MEM containing 2% FCS. Endothelial cell loss could be reduced by increasing the FCS concentration to 10%. By repute, Megacell MEM reduces the FCS dependence of cultured cells. The aim of this project was therefore to evaluate Megacell MEM for storing corneas.

**Methods** Rates of keratocyte proliferation and epithelial cell outgrowth from limbal explants in MEM or Megacell MEM were determined using Alamar blue. Paired corneas were maintained in either MEM or Megacell MEM containing 2% FCS and their endothelial cell densities were estimated over a period of 6 weeks.

**Results** The proliferation rates and viability of corneal keratocytes and epithelial cells were similar in Megacell MEM containing 2% FCS and MEM with 10% FCS. For corneas maintained in Megacell medium the initial rates of endothelial cell loss (weeks 0-3) were similar to the rates of cell loss between weeks 3-6. For corneas maintained in MEM the rates of endothelial cell loss were dependent upon pre-experimental storage time. For those stored for under 10 days the initial rates of cell loss were significantly less than the rates between weeks 3-6 and similar to those of corneas maintained in Megacell medium. For those stored for 10-16 days the initial rates of endothelial cell loss and the rates between weeks 3-6 were similar but significantly higher than those of corneas maintained in Megacell MEM. Overall the morphological appearance of the corneas in Megacell MEM was markedly better than those in MEM.

**Conclusion** Megacell MEM reduced the FCS dependence of corneal keratocytes and epithelial cells in culture and prolonged the viability of the endothelia of corneas destined for transplantation. It is therefore a better storage medium for these corneas than MEM.

## ▪ 247
## Biomechanics of Orbital Apex Fracture Following Trauma to the Malar Eminence

*TRIARIDIS S (1), KARAMPATAKIS VE (1), CONSTANTINIDIS J (2), GIGIS P (3)*
*(1) Laboratory of Experimental Ophthalmology, Aristotle University of Thessaloniki, Thessaloniki*
*(2) Otolaryngology, Aristotle University of Thessaloniki, Thessaloniki*
*(3) Anatomy, Aristotle University of Thessaloniki, Thessaloniki*

**Purpose** Following observations in trauma patients treated in our Academic ENT Unit over 20 years, we designed and performed an experimental study aiming to highlight the biomechanics of force transmission and fracture propagation to the orbital apex.

**Methods** With a specially fabricated drop weight assembly we applied single quantifiable striking force to the zygoma in 11 specimens. CT scan imaging was obtained and the specimens were examined for fractures by craniotomy and defleshing of the face. A separate control study was performed in 5 specimens to investigate the feasibility of the study.

**Results** Craniofacial fractures were recorded in 5 of the specimens. The craniofacial nature of the fractures was determined by the involvement of the sphenoid, ethmoid and frontal bones in the fracture pattern. We recorded orbital apex fractures in 4, blow out fracture of the posterior part of the orbital roof in 1 and comminuted fractures of the floor and the medial wall of the orbit with the fracture line passing in proximity or within the optic canal in 5. In half of the specimens the lateral orbital wall was comminuted and fragments were displaced in the orbit causing a decrease in the orbital volume or were impacted to the orbital apex disrupting the superior orbital fissure in 3 specimens. In 1 case the fracture line propagated to the contralateral anterior orbit.

**Conclusion** In high energy impacts, the zygoma suffers comminuted fractures and these are associated with a higher incidence of concomitant fractures at the orbital apex and the skull base. With increasing energy of impact the severity of the fractures increases and the fracture lines propagate to the skull base.

## ▪ 248
## A mouse model for Stickler's Syndrome: Ocular phenotype of mice carrying a targeted heterozygous inactivation of type II (pro)collagen gene (Col2a1)

*KAARNIRANTA K (1), IHANAMÄKI T (2), SAHLMAN J (3), PULKKINEN H (3), TAMMI R (3), LAMMI MJ (3), UUSITALO H (1), HELMINEN H (3)*
*(1) Ophtalmology, Kuopio*
*(2) Ophthalmology, Helsinki*
*(3) Anatomy, Kuopio*

**Purpose** The influences of targeted heterozygous inactivation of type II (pro)collagen gene (Col2a1) on eye structures in the 15-month-old C57BL/6JOlaHsd mouse was studied.

**Methods** Ocular histology was analyzed from tissue sections, stained with hematoxylin and eosin, toluidine blue and alcian blue. Type II collagen was localized by immunohistochemistry. Hyaluronan (HA) was stained utilizing the biotinylated complex of the hyaluronan-binding region of aggrecan and link protein (bHABC).

**Results** The anterior segment of the eye was well-formed in both genotypes, but structural defects were seen in the ciliary processes of the Col2a1+/- mice. In the lens of these mice, subcapsular extracellular matrix changes were observed. Differences in retinal structures or the number of the eyes with retinal detachment were not detected between the genotypes. In Col2a1+/- mice, staining for type II collagen was weaker in cornea, ciliary body, iris, lens, vitreous, retina, choroid and sclera than in the control mice. HA staining was detected in the extraocular tissues, ciliary body, iris and the choroid of both genotypes. HA staining was observed only in the vitreous body of the control animals.

**Conclusion** The observed structural changes in the ciliary body, lens and vitreous of the Col2a1+/- mice may represent ocular features found in the human Stickler syndrome, where the abnormalities result from COL2A1 gene mutations which lead to functional haploinsufficiency.

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## Localisation of a novel keratan sulphate stub neoepitope monoclonal antibody BKS-1 in normal human cornea, sclera and limbus

*AKHTAR S (1), KERR B (2), HAYES T (2), CATERSON B (2), MEEK KM (1)*
*(1) School of Optometry and Vision Sciences, Cardiff*
*(2) Connective Tissue Biology Laboratories, Departmant of Bioscience, Cardiff*

**Purpose** To show the use of neoepitope monoclonal antibody (MAb) BKS-1 provides a more accurate distribution of keratan sulfate (KS) in human cornea, limbus and sclera.

**Methods** MAb (BKS-1) specifically recognises a the keratanase generated neoepitope [N-acetyl-glucosamine-6-sulphate (GlcNAc-6-S)] at the non-reducing terminal of both corneal and skeletal KS GAG chains, was produced by using keratanase (from Pseudomonas sp. (EC 3.2.103)) digested "KS-peptides" from bovine cartilage aggrecan. It was used in conjunction with 5D4 to analyse KS distribution in human and bovine cornea using Western blotting, immunohistochemistry and electron microscopy

**Results** Western blot studies showed that 5D4 western blots displayed a diffuse staining pattern and it was difficult to distinguish differences between cornea, sclera and limbus. However, BKS-1 immunostaining showed a difference in KS levels, with more KS in the cornea, very low levels in the limbus and intermediate levels in the sclera. Ultrastructural studies showed that mAb BKS1 was not observed in epithelium and basement membrane labelling where as 5D4 was present in these layers. Large quantities of both antibodies were present in Bowman's layer, stroma and Descemet's membrane. But the quantity of 5D4 was significantly higher (<0.001) compared to BKS1 in all these layers of cornea.

**Conclusion** MAb 5D4 recognise all sulphated structures within one KS chain, whereas BKS-1 recognises a single neoepitope on KS after keratanase digestion. By using BKS-1, we identified a more clearly defined pattern for KS in the cornea. The presence of large quantity of antibody in the cornea suggests that KS is more prevalent in the cornea than limbus and sclera.

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## Evaluation of the In Vitro Biocompatibility of Degradable Biopolymers in Ocular Cell Cultures

*HUHTALA A (1), SALMINEN L (1), POHJONEN T (2), KAARNIRANTA K (3), UUSITALO H (3)*
*(1) University of Tampere, Medical School, Tampere*
*(2) Inion Ltd, Tampere*
*(3) University of Kuopio, Department of Ophthalmology, Kuopio*

**Purpose** Synthetic biodegradable polymers have many potential applications. Biodegradable polymer films may be used as surgical devices or implants for drug and gene delivery. In the present study, the biocompatibility of three biodegradable copolymer films (a 50:50 molar ratio of poly(DL-lactide-co-glycolide) (PLGA), a 85:15 molar ratio of PLGA, and Inion GTR membrane, a blend of 85:15 PLGA and 70:30 poly(L-lactide-co-1,3-trimethylene carbonate) copolymers in a molar ratio of 70:30) was evaluated in various ocular cell cultures.

**Methods** Biocompatibility was assessed in five cell lines (human corneal epithelial cells, rabbit stromal fibroblasts, bovine endothelial cells, human conjunctival epithelial cells, and human retinal pigment epithelial cells) by using the WST-1 proliferation and cytotoxicity test. Cells were exposed according to ISO 10993 to the extracts of biomaterials (prepared in phosphate-buffered saline at 70°C for 24 hours and diluted in medium in ratios 1:1 to 1:8).

**Results** In all cell cultures, cell viability decreased with the increasing biomaterial extract volume. The PLGA 50:50 affected viability the most. In general, the highest extraction ratio 1:1 of the PLGA 50:50 decreased viability to only few percents. The corresponding values for the PLGA 85:15 and the Inion GTR were around 45% and 70%. Corneal and conjunctival epithelial cells appeared to the most sensitive cell types, while retinal pigment epithelial cells were the most resistant.

**Conclusion** The studies suggest that the polymers tested are satisfactorily biocompatible. These biopolymer films have potential to be used as scaffolds for tissue engineering or as surgical implants, which may provide promising new therapies for ocular diseases.

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## Biological response of an oil ferrofluid in the rabbit eye

*VILLAFRUELA I, SARMIENTO B, TEJADA P*
*Ophthalmology, Madrid*

**Purpose** The present study evaluates the biological response of an oil ferrofluid in the vitreous of the rabbit eye under the influence of a magnetic field. The ferrofluid is a new material liquid, transparent and with magnetic properties. The study is a new step in the assessment of the biocompatibility in animals of the new biomaterial in order to use it in the future as a vitreous substitute.

**Methods** We inoculated 6 rabbit´s right eyes with 0.1 cc of silicone oil ferrofluid. The silicone ferrofluid was placed in the posterior chamber through the pars plana and into the vitreous. We sewed a magnet with 7/0 silk suture to the sclera. They were followed and sacrificed at different times, two of them three days later, other two seven days later and the remaining two thirty days later. We designed a control group with the same number of animals. Every animal was evaluated clinically and by optical microscopy.

**Results** We found an inflammatory shell around the area were the magnet had been placed. We found necrotic and inflammatory changes in the sclera beneath the magnet were sewed. We found free ferrofluid particles in the extracellular space as well as inside siderofagous. In those animals who were sacrificed after a three days follow-up period we found acute inflammatory changes and in the other case individuals we found chronic inflammatory changes. We did not find any change in the control animals.

**Conclusion** Silicone ferrofluids may induce an inflammatory reaction when placed in the posterior chamber, into the vitreous of the rabbit´s eye, due to the presence of free iron particles as results of the degradation of the silicone ferrofluid. Further studies are needed to confirm ferrofluid´s safety.

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## Transforming Growth Factor –ß in developing primate foetal retina

*ALLENDE A (1), NATOLI R (2), PROVIS J (2)*
*(1) Save Sight Institute, University of Sydney, Sydney*
*(2) School of Biological Sciences, Australian National University, Canberra*

**Purpose** Three transforming growth factor-ß isoforms are known to exist in mammals – TGF-ß1, -ß2, -ß3 and all have been detected in ocular tissue, suggesting a role in ocular development and homeostasis. This study investigates patterns of TGF-ß immunoreactivity and mRNA expression during primate eye development paying particular attention to the incipient fovea.

**Methods** Thirteen human eyes [13 -19 weeks' gestation (WG)] and fourteen macaque eyes [foetal day 64-postnatal 11 years] were examined. Frozen sections through peripheral and central retina were compared, by labelling with antibody to TGF-ß1, -ß2 or -ß3 and either S-opsin, RG-opsin, rhodopsin or vimentin. Sections were imaged using confocal microscopy. RNA isolated from foetal eyes was reverse transcribed to cDNA and amplified by PCR using specific primers. Amplified cDNA was inserted into pGEM-T Easy vector, cloned and used as a template for in situ hybridisation digoxigenin-labelled riboprobes. Paraffin embedded sections through the incipient fovea were analysed to identify expression pattern of mRNA for TGF-ß2 and -ß3.

**Results** RT-PCR showed that all three TGF-ß isoforms, whose identities were confirmed by sequencing, are present in the foetal eye. TGF-ß immunoreactivity and TGF-ß2 and TGF-ß3 mRNA expression was most intense in the photoreceptor layer in the foveal region and throughout the ganglion cell layer in samples studied. Sections labelled with antibodies to S opsin and RG opsin localise to sites of TGF-ß mRNA and protein production.

**Conclusion** Intense immunoreactivity and mRNA expression of TGF-ß isoforms in the photoreceptor layer of developing primate central retina, particularly in cones, suggests a specific role for TGF-ß in formation of the fovea.

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## Intraorbital optic nerve crush-induced retinal ganglion cell death. Degeneration and neuroprotection with BDNF, NT-4 or CNTF

*VIDAL-SANZ M, PARRILLA-REVERTER G, SOBRADO-CALVO P, CANOVAS I, BERNAL JM, SALINAS M, AGUILERA ME*
*Oftalmologia, Murcia*

**Purpose** To investigate in adult rats, retinal ganglion cell (RGC) survival after optic nerve (ON) crush and administration of Ciliary neurotrophic factor (CNTF), Brain derived neurotrophic factor (BDNF) or Neurotrophin-4 (NT-4), and to compare nerve fiber layer degeneration induced by intraorbital optic nerve crush (IONC) or intraorbital optic nerve transection (IONT).

**Methods** RGCs were labelled with Fluorogold applied over the superior colliculi. At 7 d, the left ON was crushed 3 mms from the optic disc. Right after IONC, rats received intravitreally vehicle (5µl of PBS), 5µg of BDNF; 5 µg of NT-4 or 5µg of CNTF. RGC survival was estimated 5, 7, 9 or 12 days after injury by counting RGCs in 12 regions of each retina. The nerve fiber layer (NFL) was examined 1, 2 or 3 months after IONC or IONT with neurofilament antibodies.

**Results** Five, 7, 9 or 12 days after IONC, the mean (±SD) densities of FG-labelled RGCs in the left axotomized retinas represented 92% (n=8), 80% (n=14), 49% (n=8) or 32% (n=11), respectively, of their contralateral intact retinas. Significant cell loss was observed between 5 and 7 days after injury. Intravitreal administration of BDNF, NT-4 or CNTF resulted 7 and 12 days later in the survival of 95% (n=7), 89% (n=7) or 91% (n=8) and 67% (n=5), 60% (n=8) or 36% (n=7), respectively. Degeneration of the NFL progressed with time and was more severe in animals with IONT.

**Conclusion** Following IONC, RGC loss appears between 5 and 7 days, and at day 12, approximately 70% of the RGC population is lost. BDNF or NT-4 protected against IONC-induced RGC loss. Degeneration of the retinal fiber layer was slower after IONC than after IONT.

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## Phototoxic retinal degeneration causes retinal ganglion cell death in adult rats

*VILLEGAS PEREZ MP, MARCO-GOMARIZ MA, HURTADO-MONTALBÁN N, BERNAL JM, VIDAL-SANZ M*
*Oftalmología, Murcia*

**Purpose** To study in normal pigmented rats whether light-induced retinal degeneration causes retinal ganglion cell (RGC) death as we have shown before in inherited photoreceptor degeneration in dystrophic Royal College of Surgeons (RCS) rats.

**Methods** The left pupil of 2 months old Lister-Hooded and pigmented non-dystrophic RCS rats was dilated with atropine and the animals were exposed to light (3000 luxes) for 72 hours and processed between 0 hours and 19 months after light exposure. Before processing, the eye fundus was inspected and in some animals, type I horseradish peroxidase (HRP) was injected intravenously to label the retinal vessels or Fluoro-Gold was applied to the superior colliculi to label RGCs. Retinas were dissected as whole mounts or processed for cross-sections, reacted for HRP demonstration or incubated with different antibodies.

**Results** All the animals presented, immediately after light exposure, a distinct arciform area of severe photoreceptor degeneration in the superotemporal retina that also showed HRP leakage and retinal pigment epithelial (RPE) cell migration. Later, complexes formed by retinal venules surrounded by RPE cells developed in this area and extended to the central retina. These complexes tractioned the inner retinal vessels, which in turn compressed the RGC axons. Animals processed over one year after light exposure showed RGC death: in pie-shaped areas of the ventral retina and in the dorsal retina, from the arciform area to the periphery.

**Conclusion** Phototoxic injury to the retina causes RGC axonal compression and death. Because these phenomena have also been observed in dystrophic RCS rats, it might be secondary to all forms of photoreceptor degeneration.

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## Quantitative analysis of retinal ganglion cells that project to the superior colliculi in adult rats and mice

*VILLEGAS PEREZ MP (1), SALINAS M (1), MAYOR S (1), HOLMES T (2), LUND RD (2), BERNAL JM (1), CANOVAS I (1), AGUILERA ME (1), VIDAL-SANZ M (1)*
*(1) Oftalmología, Murcia*
*(2) Ophthalmology and Visual Sciences, University of Utah, Utah*

**Purpose** To quantify the population and map the retinal distribution of rodent retinal ganglion cells (RGC) that project to both superior colliculi (SCi), their main target territory in the brain.

**Methods** Adult female SD rats, female and male Swiss or C57 mice, were anaesthetized and RGCs were retrogradely labelled with 3% Fluorogold applied over the surface of both SCi. One week later the retinas were dissected and prepared as whole-mounts, examined and photographed under a fluorescence microscope equipped with a digital high-resolution camera and a motorized stage connected to an image analysis system (Image-Pro Plus (IPP). The retina was imaged by adjacent, nonoverlapping frames captured in raster pattern. RGC densities were calculated from each frame by dividing GC counts by the area occupied by retinal tissue in the frame and pseudocolor density maps were constructed for representative retinas to determine higher density areas of RGCs within the retinas.

**Results** One week after FG application to both SCi the number of RGCs in adult SD rats, Swis or C57 mice were 81.710±4.8496 (mean ±SD; n=27), 47.786±3.738 (mean ±SD; n=25), or 43.906±3.375 (mean ±SD; n=19), respectively. Pseudocolor density maps from pairs of representative rat retinas indicate that high RGC density areas vary in location among different individuals.

**Conclusion** Using IPP the population of RGCs retrogradely labelled with FG from the SCi may be counted automatically with a level of confidence that is comparable to that found when RGCs are counted manually.

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## Hsp25 and Hsc70 expression in retina of trkB transgenic mice and their wild-type controls

*RANTALA JA (1), KALESNYKAS G (1), KAARNIRANTA K (1), CASTREN E (2), UUSITALO H (1)*
*(1) Ophthalmology, Kuopio*
*(2) Neuroscience Center, Helsinki*

**Purpose** Cells and tissues are constantly exposed to acute and chronic environmental stress. In order to adapt to the prevailing conditions, evolutionarily highly conserved heat shock proteins (Hsps) are expressed. They normalize homeostatic conditions in response to different kinds of stresses. Brain derived neurotrophic factor (BDNF) has been suggested to support the survival of retinal cells. Here we investigated whether mice 1) overexpressing the dominant-negative truncated splice variant of BDNF receptor trkB (trkB.T1 mice), and, in contrast, 2) overexpressing the full-length trkB (trkB.TK+ mice), affect the expression of the 25 kDa heat shock protein (Hsp25) and the 70 kDa heat shock cognate protein (Hsc70) in the mouse retina.

**Methods** Eyes from trkB.T1, trkB.TK+ mice and their wild-type controls were embedded into paraffin and cut into 5 µm sections. Sections were stained for haematoxylin/eosin, immunostained for Hsp25 and Hsc70 and analyzed under light microscopy.

**Results** The microscopical structure and immunohistochemical localization of Hsp25 and Hsc70 of transgenic and wild-type mice was identical. The strongest Hsp25 expression was seen in the retinal pigmentepithelial layer (RPE). The inner nuclear layer (INL) was only weakly stained. The Hsc70 immunostaining was moderate in the retinal ganglion cells and inner plexiform layer, but the outer plexiform layer and RPE were strongly stained. In contrast, outer nuclear layer, INL and photoreceptor cells were almost unstained for Hsc70. Cells located in the INL were immunopositive both for Hsp25 and Hsc70.

**Conclusion** Changes in the trkB receptor expression do not affect the microscopical structure or immunostaining patterns of Hsp25 and Hsc70 in the mouse retina.

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## Behaviour of glia in a model of chronic ocular hypertension

*RAMIREZ AI (1), SALAZAR JJ (1), DE HOZ R (1), ROJAS B (1), TRIVIÑO A (1), VILLEGAS-PEREZ MP (2), MAYOR-TORREGLOSA S (2), SALINAS M (2), VIDAL-SANZ M (2), RAMIREZ JM (1)*
*(1) Instituto De Investigaciones Oftalmológicas Ramón Castroviejo. UCM, Madrid*
*(2) Laboratorio Oftalmologia. Fac Medicina. Universidad Murcia, Murcia*

**Purpose** To study the effects of chronic ocular hypertension on astrocytes in adult rat retinas.

**Methods** The intraocular pressure (IOP) of the left eye of adult Sprague-Dawley rats, was chronically elevated by diode laser photocoagulation (50-100 mm, 0.4 W, 0.5 s) of the trabecular meshwork, limbus and episcleral veins .The right eyes were used as control (G0). Intraocular pressure in both eyes was measured with a Tono-Pen prior to and 1 and 2 weeks after laser treatment. For analysis of the effect of IOP level on astrocytes, glaucomatous eyes were divided into two groups: IOP 4mmHg below control (G1) and IOP 4mmHg above control (G2). Retinal whole-mounts were processed by immunohistochemistry (anti-GFAP) and divided into three zones for study: central, intermediate and peripheral. The retinal area occupied by the astrocytes was studied by computer-assisted morphometric analysis.

**Results** The retinal area occupied by astrocytes was significantly smaller in the eyes with glaucoma than in the control eyes. In glaucomatous eyes, the area occupied by astrocytes varied depending on the IOP levels: thus, the reduction of astrocytes was greater in G2 than in G1. These differences were statistically significant in the three zones analysed. Additionally, the Müller cells exhibited intense GFAP immunoreactivity in these retinas.

**Conclusion** Chronic elevation of the IOP resulted in retinal ischemia 3 weeks after laser induced IOP elevation, which induces a statistically significant loss of astrocytes in comparison with the untreated eye. The alterations of the glial cells could induce axonal degeneration.(Support: ISCIII-FIS CO3/13 and PR48/01-9903)

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## Effect of nucleotides on the activation of RPE cells by pro-inflammatory cytokines

*BOUFFIOUX C (1, 2), JUDICE DE MENEZES L (1, 2), SUAREZ-GONZALES N (2), CASPERS L (1), BRUYNS C (2), BOEYNAEMS JM (3, 2), WILLERMAIN F (1, 2)*
*(1) Centre Hospitalier Universitaire Saint-Pierre (ULB), Brussels*
*(2) Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire (IRIBHM), Brussels*
*(3) Centre Hospitalier Universitaire Erasme (ULB), Brussels*

**Purpose** RPE cells are involved in the regulation of intra-ocular inflammation (IOI). High rates of pro-inflammatory cytokines (IFNγ, TNFα and IL-1B) are found in extracellular medium during IOI. Since RPE cells possess purinergic receptors P2X (ATP) and P2Y2 (ATP=UTP), nucleotides could modulate RPE cells activation by cytokines during IOI. The aim is to study the effect of nucleotides on the activation of RPE cells by pro-inflammatory cytokines.

**Methods** RPE cells were stimulated by IFNγ, TNFα or IL-1B in the presence or absence of nucleotides(ATPgS, UTP or UDP). Stat-1 and IKB phosphorylation were studied by Western Blot. MHC class I, class II, ICAM-1 and VCAM-1 expressions were analysed by flow cytometry.

**Results** Nucleotides did not have any effect when used alone. TNFα activated IKB and upregulated MHC class I and ICAM-1 expressions. IFNg activated Stat-1, induced MHC class II and upregulated MHC class I and ICAM-1 expressions. None of the nucleotides tested had any effect on the IFNg pathway. UDP inhibited MHC class I expression induced by TNFα. ATPgS and UTP seemed to increase IL-1B induced IkB phosphorylation.

**Conclusion** Nucleotides had no effects on IFNg activation of RPE cells. On the contrary, UDP seemed to modulate RPE cells activation by TNFα, while ATPgS and UTP could act positively on IL-1B pathway. Further work must be done to characterise the role of nucleotides in RPE cells activation during intraocular inflammation.

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## TGF-ß protects RPE via an increase of Ser-59 phosphorylated alphaB-crystallin

*YU AL (1), ALGE CS (1), KAMPIK A (1), BLOEMENDAL H (2), WELGE-LÜßEN UC (1)*
*(1) Ophthalmology, Munich*
*(2) Biochemistry, Nijmegen*

**Purpose** In the retinal pigment epithelium (RPE) of the macular region the expression of alphaB-crystallin is increased compared to the periphery. The chaperone activity of αB-crystallin depends on the phosphorylation of specific serine residues (Ser-19, -45, -59). In the pathogenesis of early age-related macular degeneration (AMD) various stressors like oxidative stress and an increased expression of transforming growth factor-beta 2 (TGF-ß2) are discussed. In the present study the influence of oxidative stress and TGF-ß2 on the expression and phosphorylation of alphaB-crystallin and its specific serine residues was investigated.

**Methods** Monolayer cultures of human RPE cells were treated with TGF-ß2, or stressed by oxidant-mediated injury. Induction of alphaB-crystallin and the corresponding mRNA was assessed by Western and Northern blot analyses. The site specific phosphorylation of alphaB-crystallin was determined by antibodies recognizing the specific phosphorylated serine residues (Ser-19, -45, -59) of human alphaB-crystallin.

**Results** An increase of alphaB-crystallin mRNA and protein expression in RPE cells was observed after exposure to oxidative stress and TGF-ß2. Both treatments significantly increased the phosphorylation of alphaB-crystallin. Site specific phosphorylation of Ser-59 was more intense following TGF-ß treatment. The other investigated Ser residues (Ser-19 and -45) were equally induced by both stimuli.

**Conclusion** This is the first time to show that alphaB-crystallin is not only induced by stress factors but also by TGF-ß2 in RPE cell cultures. The increased expression and site specific phosphorylation of Ser-59 of alphaB-crystallin by TGF-ß2 might protect RPE cells against stress in the early stages of AMD.

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## Failure of Limited Proteolysis of Pigment Epithelium-Derived Factor (PDEF) in Tenon's Capsule of Patients with Progressive Myopia

*LAZUK AV (1), IOMDINA EN (1), KOSTANYAN IA (2), ZHOKHOV SS (2)*
*(1) Helmholtz Research Institute of Eye Diseases, Moscow*
*(2) Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow*

**Purpose** Progressive myopia is accompainied by dystrophic processes in the eye (sclera and retinal dystrophies). Metabolic disorders of scleral fibroblasts are probably associated with pathological changes in retinal pigment epithelium. PEDF is a neurotrophic factor responsible for normal retinal development; is affinity to glycosaminoglycans and collagens I and III was schown as well. Since Tenon's capsule and scleral fibroblasts are practically identical, they probably undergo the same pathological changes in progressive myopia. Purpose: comparsion of PEDF content in Tenon's capsules in progressive myopia and hyperopia

**Methods** Tenon's capsule samples were obtained from 42 patients ageg 6-28 (16 with moderate, 16 with high myopia; each group included 9 patients with peripheral retinal disorders; 10 with hyperopia) during scleroplasty and surgeries for squint. PEDF content was determined in sample homogenates by Western blot analysis using polyclonal antibodies against 345Lys-366Glu PEDF molecule fragment.

**Results** A band corresponding to the full-length PEDF molecule (~50kDa) was detected in all samples. In hyperopia, its average intensity higher, and another band (~45 kDa) was revealed as well. This band was at least ten times weaker in 50% of patients with moderate, and in 69% of high myopia. In complicated myopia, this band was more intensive than in non-conplicated.

**Conclusion** A limited proteolysis of PEDF occurs in Tenon's capsule in hyperopie, which is supressed in myopia. The only bond 382Leu-383Thr appears to be hydrolyzed, which is the primary target of serine proteases. Our results show that Tenon's capsule as a valuable object of myopia pathogenesis studies during lifetime.

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### Expression of endostatin in human choroidal neovascular membranes secondary to age related macular degeneration

*TATAR O (1), ADAM A (2), ROHRBACH J Martin (1), LUCKE K (3), SHINODA K (4), BARTZ-SCHMIDT KU (1), GRISANTI S (1)*
*(1) Eberhard-Karls University, Eye Clinic Department 1, Tuebingen*
*(2) Eberhard-Karls University, Department of Pathology, Tuebingen*
*(3) Eye Clinic Universitätsallee, Bremen*
*(4) Keio University, Department of Ophthalmology, Tokyo*

**Purpose** To investigate the expression of endostatin, an endogenous angiogenesis inhibitor, in human choroidal neovascular membranes (CNV) secondary to age related macular degeneration (AMD) with regard to vascularization and proliferative activity.

**Methods** Retrospective review of 36 patients who underwent removal of CNV. Thirty-six CNV were analyzed by light microscopic immunohistochemistry for CD34 (endothelial cells, EC), CD105 (activated EC), Ki-67 (cell proliferation), Cytokeratin 18 (epithelial cells), VEGF (vascular endothelial growth factor), E-selectin and endostatin. Donor eyes (n=7) including one with AMD were used as controls.

**Results** Endostatin immunostaining was present in choroidal vessels of five and in RPE cells and Bruch's membrane of two donor eyes without AMD. In the eye with AMD, endostatin was present in RPE, Bruch's membrane and choroidal vessels. Ninety-two percents (33/36) of CNV disclosed endostatin staining. RPE cells, choroidal vessels and stroma were positive in 50% (16/36), 72% (26/36), and 78% (28/36) of the membranes respectively. Both control eyes and CNV expressed all the investigated markers except E-selectin being positive only in membranes.

**Conclusion** Endostatin, an endogeneous angiogenesis inhibitor, is expressed in CNV. It is co-expressed with E-selectin that is required for its antiangiogenic activity only in CNV but not in control eyes. Therefore, endostatin may contribute to the involution process of the neovascularization in AMD.

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### Ultrastructural analysis of photoreceptor neurogenesis in the adult monkey and human far peripheral retina

*CUENCA N (1), MARTINEZ-NAVARRETE G (1), MARTIN-NIETO J (2), ANGULO A (3)*
*(1) Biotecnología, Alicante*
*(2) Fisiología, Genética y Microbiología, Alicante*
*(3) Interuniversitario de Óptica, Alicante*

**Purpose** In vitro, retinal stem cells and differentiating neurons have been identified in neurosphere cultures from the ciliary body epithelium of adult rodents and humans. We have examined at the ultrastructural level cones and rods residing in the far peripheral retina of adult primates, in order to determine whether they represent either differentiating or degenerating photoreceptors.

**Methods** Transmission electron microscopy was carried out on ultrathin sections of monkey peripheral retina. As well, cryostat vertical sections obtained from adult macaques and humans were subjected to immunostaining with specific antibodies against nestin and a set of phenotypic markers for rods and cones.

**Results** Cones and rods located at the peripheral retinal margin exhibit an immature ultrastructure, with tiny outer segments and a huge axon terminal. De novo formation of outer segment disk membranes at the upper portion of incipient ellipsoids was apparent from the fusion of Golgi vesicles. in the non-laminated retina. Along this zone, the sequential morphological development of cells expressing photoreceptor markers was accompanied by the establishment of synaptic contacts with horizontal and bipolar cells, in parallel to the growth in length of outer segments. However only two horizontal-cell processes could be identified at ribbon triad synapses.

**Conclusion** The far peripheral retina thus constitutes a region where neurogenesis of cone and rods takes place in a spatial-temporal fashion in adult primates. This process can be envisioned to have a role in the normal turnover of retinal circuitries that become lost along life in mammals.Support: MCyT BFI2003-01404, GV04B/452, ONCE and Fundaluce.

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### Alpha-Synuclein-containing synaptic ribbons, outer segment disks and desmosome-like junctions in the primate retina

*MARTINEZ-NAVARRETE G (1), ANGULO A (2), HERRERO MT (3), MARTIN-NIETO J (4), CUENCA N (1)*
*(1) Biotecnología, Alicante*
*(2) Interuniversitario de Óptica, Alicante*
*(3) Anatomía Humana y Psicobiología, Murcia*
*(4) Fisiología, Genética y Microbiología, Alicante*

**Purpose** Alpha-Synuclein is a presynaptic protein postulated to have a role in synapse maintenance and neural plasticity and neurodegenerative disorders. We have analyzed alpha-synuclein distribution in the distinct neuronal types of the retina, in order to obtain clues on its physiological role in the retina.

**Methods** We have used ABC preembedding immunocytochemical methods for electron microscopy and cryostat vertical sections of retinas from adult monkeys and humans for fluorescence microscopy. Immunostaining was carried out using with antibodies against alpha-synuclein and other markers specific for the distinct retinal neuronal types.

**Results** A strong immunoreactivity was found in the outer and inner plexiform layers. This protein immunolocalized to the cell bodies and dendrites from bipolar and amacrine cells. Ultrastructurally, alpha-synuclein located at desmosomes established between photoreceptors and Müller cells lining the outer limiting membrane. Immunostaining was also present in the outer segment disks from photoreceptors. At the level of pedicles and spherules alpha-synuclein was identified at synaptic ribbons and desmosome-like junctions between horizontal cells in the OPL.

**Conclusion** Alpha-Synuclein localization in photoreceptors suggests an involvement of this protein in outer segment disk formation and synaptic transmission at the OPL level. Roles in desmosome-like junctions, in the formation of the outer limiting membrane and/or the turnover of presynaptic vesicles can also be postulated. It is thus possible that mutations in the alpha-synuclein gene could be causative of retinosis pigmentosa. Support: MCyT BFI2003-01404, GV04B/452, ONCE and Fundaluce

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### Mediation of Ghrelin's relaxing effect on iris sphincter and dilator muscles

*ROCHA DE SOUSA A (1, 2), SARAIVA J (1), FARIA PA (2), FALCÃO REIS F (2), LEITE-MOREIRA A (1)*
*(1) Physiology, Fac Medicine University of Porto, Porto*
*(2) Ophthalmology S Joao Hospital, Porto*

**Purpose** Ghrelin is a 28 aa peptide with important functions in smooth, cardiac and skeletal muscles. Some of its muscular effects seam mediated by a receptor other than GHRS–1a. The aim of this study was to investigate the mediation of its ocular effects by the GHSR–1a receptor in rat and rabbit iris sphincter or dilator muscle.

**Methods** Rabbit, Wistar rat iris sphincter muscles and rabbit iris dilator muscle were dissected, mounted on a vertical organ (modified Krebs–Ringer; 1.8 mM Ca2+; 35ºC) attached to a force transducer and precontracted. The effects of 1–5,Ghrelin (Gr, 10e–9– 6*10e–5M) on rabbit sphincter (n=7), dilator (n=6) and rat sphincter(n=6) muscle were evaluated. On rabbit sphincter that effect was also tested in the presence of i) L-NA (10e-4M; n=14); ii) indo (10e-5M; n=14); iii) DLysGHRP6 (10e-4M; n=12). Finally, the effect of 1–5,des–octanoil Ghrelin (DGhr; 10e–9– 6*10e–5M) on rabbit sphincter (n=7), dilator (n=6) and rat sphincter (n=6) muscles were evaluated.

**Results** Ghrelin promoted a concentration-dependent relaxation of the rabbit sphincter (34.1±12.1%), dilator (25.8±5.0%) and rat sphincter (63.3±7.1%) muscles maximal at 6*10e-5 M. On rabbit sphincter there was a trend for L-NA to exacerbate (58.0±10.5%) and for Indo to attenuate (20.3±12.1%) ghrelin's relaxing effect. DLysGHRP6 does not inhibit that relaxing effect (118.1±21.1%). DGr promoted also a similar relaxation of rabbit (43.4±5.2 %) and rat (60.5±42.2 %) sphincter. However in the rabbit dilator DGr did not have effect (–3.1±12.1 %).

**Conclusion** Ghrelin relaxation is mediated by GHSR1a in iris dilator but not in iris sphincter muscles.

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### The ß-defensin HBD2 induces specific patterns of Ca2+ responses in conjunctiva cells (IOBA-NHC) associated with activation of L-type channels

*MERGLER SM (1), SCHLORF TS (2), RECKER KR (2), PLEYER U (1), DIEBOLD YD (3), PAULSEN FP (2)*
*(1) Dept. Ophtalmology, Charité - University Medicine Berlin, Berlin*
*(2) Anatomy and Cell Biology, Martin-Luther-University Halle-Wittenberg, Halle*
*(3) Applied Ophthalmobiology, University of Valladolid, Valladolid, Valladolid*

**Purpose** Corneal and conjunctival epithelial cells are constanly exposed to potential pathogenic agents such as infectious organisms. Recently, antimicrobial peptides such as ß-defensins (human ß-defensins 1-3, HBD1-3) that are induced after microbial contact have been shown to be effective in the protection of the ocular surface against distinct bacteria. However, the expression and regulation of HBD1-3 at epithelial surfaces are not clearly known. Interestingly, Ca2+ triggers the expression of HBD2 in human keratinocytes. Aim: Characterization of the effects of HBD2 on intracellular Ca2+ concentration ([Ca2+]i) in a permanent conjunctiva cell line (IOBA-NHC).

**Methods** The effects of recombinant HBD2 were determined in fura2-loaded IOBA-NHC cells with a fluorescence video imaging system.

**Results** Ca2+ responses in IOBA-NHC cells were clearly detectable in a Na- and K-free solution. Specifically, extracellular application of HBD2 (100 ng/ml) increased [Ca2+]i to a peak level of 109 ± 1 % of control (set to 100 %) followed by a recovery to the baseline after 5 min (± SEM; n = 5). In the presence of the L-type channel blocker nifedipine (5 µM), [Ca2+]i significantly decreased to 80 ± 4 % of control (n = 4; $p < 0.0001$).

**Conclusion** Conjunctiva cells display Ca2+ patterns in responses to HBD2 depending on voltage-dependent L-type Ca2+ channel activity. This could play an important role in the expression and regulation of the ß-defensin HBD2 in the conjunctiva. Further investigations are currently being performed to better understand the effect mechanisms of HBD2.Supported by Wilhelm Roux program grant FKZ 09/16, Sicca-research-support and DFG PL/150-14 (in part)

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### The role of histology in the diagnosis of a slipped extraocular muscle

*VOROS GM, RESSINIOTIS T, STRONG NP*
*Royal Victoria Infirmary, University of Newcastle upon Tyne, United Kingdom., Newcastle upon Tyne*

**Purpose** To highlight the role of histology in the differential diagnosis between 'slipped' and 'detached' extraocular muscle.

**Methods** We examined a 24-year-old male with a longstanding left esotropia. He reported left squint surgery at the age of three due to esotropia. Orthoptic assessment confirmed a 50 D left esotropia. Abduction of the left eye was totally absent. Differential diagnosis included 'slipped' and 'detached' left lateral rectus muscle. Surgical exploration followed.

**Results** Forced duction test revealed that there was no mechanical restriction of abduction. Surgical exploration of the left lateral rectus muscle revealed an empty tendon capsule attached at 8mm from the corneal limbus. The capsule was opened, and probable fine muscle fibers were identified at 21mm from the limbus. The empty capsule was resected, and the presumed muscle fibers were advanced and attached to the sclera at 8mm from the limbus. Intra-operatively the surgeon felt that the muscle had not been identified, however pathology examination of the resected capsule revealed muscle fibers at its posterior edge. Thus, the left lateral rectus muscle had just 'slipped' and not been completely 'detached' Postoperatively, the patient had a 6 D residual esotropia, which remained unchanged over a 12 month follow up period.

**Conclusion** Histology can differentiate between 'slipped' and 'detached' extraocular muscle, when surgical exploration is inconclusive.

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### Effectiveness of enhanced medial rectus recession with looped sutures

*RESSINIOTIS T (1), VOROS GM (2), KNOWLES E (3), TIFFIN PAC (1), ALLCHIN RW (1)*
*(1) Department of Paediatric Ophthalmology and Strabismus, Sunderland Eye Infirmary, Sunderland*
*(2) Department of Ophthalmology, Royal Victoria Infirmary, Newcastle upon Tyne*
*(3) Departments of Orthoptics, Sunderland Eye Infirmary, Sunderland*

**Purpose** Management of large residual deviations following previous 'maximal' medial rectus (MR) recession or of large near angles may be problematic. We report our experience with the use of augmented MR recessions using looped sutures in a group of patients with at least 2 years follow-up.

**Methods** All patients who had enhanced MR recession using a looped suture technique performed at Sunderland Eye Infirmary between 1988 and 2002 were identified. A retrospective analysis of the medical records was made with particular reference to diagnosis, previous strabismus surgery and pre-and post-operative measurements. Patients were then invited to return for a contemporary orthoptic assessment and asked to complete a short questionnaire.

**Results** Twenty patients were identified. Ten patients agreed to return for orthoptic assessment. One patient who did not reply but with 8 years documented follow-up and one patient now deceased but with two years documented follow up were also included. Follow-up ranged from 2 to 16 years (mean 8.6 years).Early post-operative alignment was good with 12/17 patients aligned within 12 PD of orthophoria for near. 4/12 patients had stable alignment associated with good binocular function. 6/12 showed stable long-term alignment with no significant exotropic drift despite having no binocular function. One patient developed an immediate post-op consecutive XT with diplopia requiring further surgery. Only one patient showed significant long-term exotropic drift. All patients were satisfied.

**Conclusion** Augmented recession of the MR using looped sutures is a useful technique. Postoperative alignment rate and patient satisfaction both appear high.

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### Variation of distance and near lateral phorias along the morning

*SOBRADO-CALVO P, VIDAL LÓPEZ J*
*(1) Ophthalmology and Optometry - University of Murcia, Murcia*

**Purpose** The objective of this study was to assess if distance and near lateral phorias varies along the morning in High School students.

**Methods** Distance and near lateral phorias were evaluated in 194 (114 males and 80 females) High School students using two differents methods of measurement: Bioptor Vision Test (Stereo Optical, Co) and von Graefe method using prisms bar. Measurements were registered from 8:00 to 14:00 hours. Regression slopes between hour in the morning and phorias values were computed. Values were analysed using an statistical sofware (SPSS v.11).

**Results** Distance lateral phorias varied towards endophoric values significantly along the morning, when they were measured with the Bioptor Vision Test (b=-0.19, t=-2.08, p=0.04). When distance lateral phorias were measured with the prisms bars, the results also tend to endophoric values, although these changes were not significants (b=0.09, t=0.57, p=0.57). Likewise, near lateral phorias changed towards endophoric values along the morning both in Bioptor Vision Test (b=-0.19, t=-2.09, p=0.04) as in prisms bars (b= 0.48, t=2.49, p=0.01).

**Conclusion** Lateral phorias changed towards endophoric values in terms of the hour of the morning. These variations were recorded by means of two methods: Bioptor Vision Test and von Graefe with prisms bar; however, prisms bar did not show significant differences in distance lateral phorias, probably because this technique biases the little values of endophoria or exophoria towards ortophoric values (Sobrado y Vidal, 2005).

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## Changes in fusional vergences in High School students along the morning

*VIDAL LÓPEZ J, SOBRADO CALVO P*
*Oftalmología, Optometría, Otorrinolaringología y Anatomía Patológica, Murcia*

**Purpose** To investigate if there are variations in distance and near fusional vergences along the morning with students daily tasks.

**Methods** Distance and near fusional vergences (positives and negatives, break and recover) were recorded in 165 High School students (103 males and 62 females) with horizontal prisms bar. Measurements were recorded at different hours in the morning (between 8:00 and 14:00 hours). Regression slopes were computed for both distance and near, positive and negative vergences (break and recover). Statistical sofware (SPSS v.11) was used to analize all the results.

**Results** Positive fusional vergences at distance did not show significant differences along the morning, neither for break values (b=-1.13, t=-1,65, p=0.10) nor for recover values (b=-0.25, t=-0.74, p=0.46). Negative fusional vergences at distance did not vary significantly along the morning (break: b=-0.61, t=-1.31, p=0.19; recover: b=0.12, t=0.80, p=0.43).Likewise, near positive fusional vergences did not change significantly (break: b=-0.36, t=-0.82, p=0.41; recover: b=-0.09, t=-0.31, p=0.76).In the case of negative fusional vergences at near, there were not significant changes (break: b=-0.34, t=-0.98, p=0.33; recover: b=-0.08, t=-0.40, p=0.69).

**Conclusion** Daily tasks do not induce significant changes in fusional vergences at distance and near in High School students.Recently, we documented that student daily tasks affect distance and near lateral phoria degree (Vidal-López and Sobrado-Calvo, 2005), but the measurement with prisms bar infravalue small changes in the alignement of visual axis (Sobrado-Calvo and Vidal-López, 2005). We believe that results obtained in fusional vergences in the present study could be affected by the measurement with prisms bar.

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## Comparison of the measurements of lateral phorias with the Bioptor Vision Test and the von Graefe method

*SOBRADO-CALVO P, VIDAL LÓPEZ J*
*Ophthalmology and Optometry - University of Murcia, Murcia*

**Purpose** To investigate the correlation between the values of distance and near lateral phorias obtained by the von Graefe method and the Bioptor Vision Test (BVT-Stereo Optical, Co), and to establish a numerical value that could be asigned as normal value for lateral phorias with the BVT.

**Methods** In 130 subjects, students in a Primary School, we measured distance and near lateral phorias by means of the von Graefe method with prisms bar and the BVT. All the results were analyzed using the statistical sofware SPSS, v.11.

**Results** The mean values obtained with von Graefe method were 1.5±2.8 and 4.3±4.5 base in prism diopters, for distance and near lateral phoria respectively. The results obtained with the BVT were the mean numeric values of 5.3±2.2 and 6.4±2.3, for distance and near lateral phoria respectively. There was a statistical correlation between the two methods for distance lateral phoria (p=0.001), but not for the near one (p= 0,216).Values showed a more normal distribution with BVT than with the von Graefe method, because it was observed a tendency to the grouping of values around the central one for the last method.

**Conclusion** For the BVT, the numeric value that corresponds with the normality both for distance and near lateral phorias must be between 5 and 6±2.The measurement with the BVT produces a tendency towards esophoria, both at distance and near phorias. On the contrary, the measurement with the von Graefe method induces a tendency towards exophoria. The BVT is a more secure system to evaluate lateral phorias, because the distribution of the results is normalized, unlike occurs with the von Graefe method.

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## Effects of orthoptic treatment on the CA/C and AC/A ratios

*BRAUTASET R (1), JENNINGS A (2)*
*(1) Unit of Optometry, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm*
*(2) Faculty of Life Sciences, The University of Manchester, Manchester*

**Purpose** To evaluate the effect of orthoptic treatment on the AC/A and CA/C ratios in subjects with convergence insufficiency (CI).

**Methods** The change in AC/A and CA/C ratios after a 12-week period of home-based orthoptic treatment was examined in 10 subjects (mean age 25.4 years ±4.1 SD). Both the AC/A and CA/C ratios were measured using gradient response methods. For the AC/A ratio the gradient phoria method was used and for the CA/C ratio the prism induced change in accommodation was measured using a PowerRefractor.

**Results** No change in the AC/A and CA/C ratios ($p > 0.05$) could be found after orthoptic treatment. However, improvements were found ($p < 0.05$) in the fast and slow vergence mechanism.

**Conclusion** Despite improvements in the fast and slow vergence mechanism no change could be found in the AC/A and CA/C ratios after orthoptic treatment in CI subjects. The results therefore indicate that the AC/A and CA/C ratios are constant parameters of the vergence system in non-presbyopic subjects. However, this is unexpected in the light of the present understanding of CI and in light of current accommodation and vergence control models.

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## The effect of maintenance therapy on amblyopia recurrence following successful primary treatment

*SJÖSTRAND JB, NILSSON J, BAUMANN M*
*Institute of Clinical Neuroscience, Göteborg*

**Purpose** To evaluate the effect of maintenance therapy on amblyopia recurrence following successful primary treatment

**Methods** In a prospective study on the results of treatment for amblyopia due to strabismus or anisometropia at our department, 35 subjects were successfully treated (22 subjects with strabismus and 13 subjects with straight-eye amblyopia). All children where put on maintenance therapy(patching 3 to 7 hours a week,Ryserfilter attached to glasses or infrequent instillation of atropine drops ) and regularly examined up to at least 8 years of age.

**Results** Six cases deteriorated >= 0.2 logMAR, which was considered recurrence of amblyopia. Two of these cases had a second recurrence. Notably, all six subjects presenting with recurrence had microstrabismus, and presence of strabismus was found to increase the risk of amblyopia recurrence ($p < 0.05$). All but one of the recurrences appeared within 6 months after cure. Age at cure or initial interocular difference of visual acuity did not affect the risk of recurrence.

**Conclusion** Recurrence of amblyopia is common, even in subjects with maintenance therapy. Strabismus might increase the risk of recurrence, possibly due to persisting suppression.

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## Peculiarities of pupillogrames in healthy persons

*BOYCHUK IM (1), BUSHUYEVA NN (1), SLOBODYANIK SB (2), DUHAIR S (1)*
*(1) Lab.of Binocular vision disturbances, Odessa*
*(2) Lab. of functional methods of investigation, Odessa*

**Purpose** The investigation was performed to reveal the indices of pupillograms which can characterize the state of pupill-accomodative-convergence system response in healthy persons watching near objects.

**Methods** 17 patients without complaints aged 17–19 were examined on the elaborated pupillography device. Except pupillography the following visual functions were investigated: visual acuity, refraction, reserves of accomodation, mezopic vision. The balance of autonomic nervous system was appreciated by Cerdo index. With the help of elaborated device and corresponding software for pupillography method the changes of pupil parameters during investigation of direct and consensual reaction on flashlight test and accommodative-convergence response during watching by person fixed object were conducted

**Results** The temporal phases have been found for characterizing pupil states when applying an accommodation-convergence stimulus. There were defined new indices of functional state and pupil reaction under effect of stimulus, resulting in accommodation-convergence pupil response (phase duration in seconds, area change amplitude, anisocoria, pupil area fluctuations number and frequency with respect to phases), which correlated with accommodation- convergence pupil response. Significant correlations (Rs > 0,7) were found between pupil reactions and certain visual functions, among them: visual acuity, mezopic vision and accommodation.

**Conclusion** Practical importance of pupillogrammes data in the healthy persons is its usefulness for diagnostics of functional state of autonomic innervations of the eyes, in patients with disturbances of accommodation and convergence to choose adequate medical treatment

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## Evaluation of optic disc edema using OCT and HRT

*KAMPPETER BA, JONAS JB*
*Department of ophthalmology, Faculty for clinical medicine Mannheim, University of Heidelberg, Mannheim*

**Purpose** Detailed follow up of the extend of optic disc elevation is essential for assessing possible progression of disease involving optic disc swelling. New laser imaging techniques like OCT and HRT produce optic nerve head images which can be measured directly in absolute or relative values. Compared to the multiple two dimensional planes by HRT, OCT is able to obtain real cross sectional images. In addition, OCT´s fixed reference structure is the pigmentepithelium and thus not user dependend like the HRT´s contour line. This poster wants to outline possible advantages of OCT compared to HRT in follow up diagnosis of optic disc swelling.

**Methods** Thr right eye of a 32 year old female patient, diagnosed with papille edema due to elevated intracerebral pressure, was measured 5 times during a 2 week period by HRTII and OCT3. To reach a reproducible reference plane in HRT evaluation, an unconventional contour line was drawn off center of the optic disc (diameter0.4mm). Measurements were done in interaktive measuring mode. In OCT image a distance from pigmentepithelium´s end to the highest point of point of nerve fiber layer was taken on both sides of the neuroretinal rim.

**Results** The temporal side of the optic disc, measured an a horizontal line centered on the optic disc, showed an increase of elevation of 10,8% (HRT) and 12% (OCT). Increase in nasal sector was 4% by HRT compared to 7% by OCT.

**Conclusion** OCT and HRT showed equivalent results. One has to take into account, that setting of a correct contour line as measurement´s basis in HRT imaging is especially problematic in edematour optic discs. OCT offers due to its real cross section imaging and steady reference structure a possible easier and less prone to error measuring technique.

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## Sleep apnea, the most frequent disorder associated with Nonarteritic Anterior Ischemic Optic Neuropathy

*RENARD ER (1), PALOMBI KP (1), PEPIN JLP (2, 3), LEVY PL (2, 3), DECHAUD CD (2, 3), CHIQUET CC (1), MOUILLON MM (1), ROMANET JPR (1)*
*(1) Ophthalmology, Grenoble*
*(2) Sleep Laboratory and EFCR, Grenoble*
*(3) H2P Laoratory, INSERM ESPRI EA 3745, Grenoble*

**Purpose** To evaluate newly diagnosed Nonarteritic Anterior Ischemic Optic Neuropathy (NAION) patients for the existence of sleep apnea.

**Methods** Polysomnography allowed determining the prevalence rate of sleep apnea in NAION patients. The prevalence found in the study was compared to historical controls. Hypertension, diabetes, hyperlipidemia, atheromatous lesion of carotid vessels and inflammatory diseases being all classical risk factors associated with NAION were also identified.

**Results** Twenty-seven consecutive newly diagnosed NAION patients (18 men, mean age 65 years) were included in the study. Eighty-nine percent of the patients exhibited a sleep apnea syndrom (Respiratory disturbance index: 37.2+/-18.3/h). Risk ratio for a NAION patient to suffer from sleep apnea was 4.9 compared to historical controls ($p<0.001$). Sleep apnea was 1.5 to " fold more frequent than the rate of the other identified risk factor classically associated with NAION. Continuous positive airway pressure treatment in 70% of the patients.

**Conclusion** Sleep apnea is the most frequnet disorder associated with NAION and should be systematically investigated in this population. The association beetween both diseases may be explained by similar pathophysiological mechanisms.

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## A flawless MRI can leave you with a headache - Is B-Scan Ultrasonography superior to CT and MRI?

*NESTEL AR, WHITTAKER KW, GIBSON RA*
*North Devon District Hospital, Department of Ophthalmology, Barnstaple*

**Purpose** To report greater sensitivity of orbital B-scan Ultrasonography relative to orbital CT and MRI

**Methods** B-Scan ultrasonography, case-reportBackground: A 33 year old caucasian female with a six month history of therapy resistant left hemicranial headache of unknown aetiology was referred to our department by a neurologist. CT and MRI-scans of the brain and orbits had detected no abnormality.

**Results** Ultrasonographic assessment revealed an incompressible, retrobulbar structure superonasally in the left orbit. The internal reflectivity was low and homogenous – both parameters being compatible with flowing blood. The location and incompressibility were compatible with an arterialised flow in the superior ophthalmic vein.In context with the clinical picture, the presumptive diagnosis of a low-flow Carotid-Cavernous-Sinus-Fistula (low-flow CCF) could be made.

**Conclusion** Low-flow CCF may remain undetected for many months (1,2). Symptoms are atypical and may be misleading (3,4). Signs on MRI such as an enlarged posterior intercavernous sinus may be subtle and are sometimes only found retrospectively (5). Findings on B-scan ultrasonography are obvious and unequivocal (6,7). B-scan ultrasonography in the presented case displayed a higher sensitivity than CT and MRI in picking up a low-flow CCF. In the setting of an unilateral headache in association with a chronic red eye, B-scan ultrasonography appears to be an ideal diagnostic tool – it is sensitive, risk-free and inexpensive.

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## Two year's training of homonymous hemianopia with flicker stimulation

*RANINEN AN (1, 2, 3), HENRIKSSON L (4, 5, 6), VANNI S (4, 5, 6), HYVÄRINEN LVM (7), NÄSÄNEN R (8)*
*(1) University of Helsinki, Helsinki*
*(2) Bio and Environmental Sciences, Helsinki*
*(3) Physiology, Helsinki*
*(4) Helsinki University of Technology, Espoo*
*(5) Low Temperature Laboratory, Espoo*
*(6) Brain Research Unit, Espoo*
*(7) Lea-Test Ltd, Helsinki*
*(8) Finnish Institute of Occupational Health, Helsinki*

**Purpose** As a result of one-year training, the flicker sensitivity on the hemianopic side became equal to that of the intact side up to an eccentricity of 20 deg. Now, we wanted to find out, if continued training could further extend the area of equal sensitivity to even larger eccentricities. Hence, both men continued training for another year.

**Methods** Subjects KS and IT participated in two weekly training sessions, where a flickering disk was detected at eccentricities 30 (KS), 40 and 60 degrees (IT) along the horizontal meridian. Flicker frequencies were 1, 2, 5, 10, 15, 20, and 35 Hz. Neuromagnetic responses were followed during rehabilitation, and IT's fMRI activation to blind field stimulation was recorded after rehabilitation.

**Results** After 17 months of training the flicker sensitivities of subject IT became symmetric at the eccentricity of 40 deg. After an additional 5 months of training his flicker sensitivities in the hemianopic visual field at the eccentricity of 60 deg became nearly equal to that of the intact visual field. The only exception was the lower right quadrant, where sensitivities were lower than those of the intact side. In the end of the 2nd year of training flicker sensitivities of KS at 30 deg were symmetric. During training both IT's and KS's MEG recordings showed new evoked responses to hemianopic visual field stimulation. IT's fMRI showed that the two visual fields were represented in one set of retinotopic areas.

**Conclusion** Our findings prove that there is still plenty of plasticity in the brain at the age over 60-years. This work shows also that field loss in homonymous hemianopia can be rehabilitated with long-term flicker stimulation of the blind hemifield.

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## Reducing vascularity in complex anterior segment surgery with topical apraclonidine 1%

*SII F, LOCKWOOD A, SHAH P, TODD B*
*Ophthalmology, Birmingham*

**Purpose** To describe the additional use of topical apraclonidine 1% for the preoperative reduction of the anterior segment vascularity.

**Methods** A single topical dose of apraclonidine (an alpha-2 agonist) 1% is applied as the patient enters the operating theatre for anterior segment surgery such as trabeculectomy, bleb needling revision, tube drainage surgery, cyclodialysis, cleft repair and bleb reconstruction.

**Results** The success of anterior segment filtration surgery can easily be compromised by the vascularity of this region, both by influencing the decision of surgical approach and the leak of its profibrotic products within the filtration pathway being created. The routine application of a single topical dose of apraclonidine 1% prior to surgery has a profound and rapid effect on reducing conjunctival, anterior ciliary and iris vessel calibre. This minimises the risk of intraoperative bleeding into the surgical field. Other agents such as adrenaline can cause a rebound hyperaemia and undesirable pupil dilation.

**Conclusion** The preoperative application of topical apraclonidine 1% has been found to allow a much safer and more extensive conjunctival dissection and scleral trapdoor creation for bleb formation with excellent haemostasis.

## ▪ 279
## A new biodegradable polymer for triamcinolone delivery

*EPERON S (1), FELT-BAEYENS O (2), MORA P (3), LIMAL D (4, 5), SAGODIRA S (6, 5), BRETON P (7, 5), GURNY R (2), GUEX-CROSIER Y (1)*
*(1) Jules Gonin Eye Hospital, University, Lausanne*
*(2) School of Pharmacy, University, Geneva*
*(3) Institute of Ophthalmology, University, Parma*
*(4) OM Pharma, Geneva*
*(5) Virsol, Paris*
*(6) Fist S.A., Paris*
*(7) IDM, Paris*

**Purpose** To develop a biodegradable scleral implant delivering a corticosteroid within the eye for many weeks and made of poly(methylidene malonate) or PMM2.1.2.

**Methods** Scleral discs were manufactured by a compression-molding method using PMM2.1.2 and plasticizers. Implants were fastened to the sclera of anesthetized rabbits. Biocompatibility of unloaded implants was assessed by 1) the total clinical score (=sum of conjunctival hyperemia, chemosis and edema) and 2) inflammation parameters (=inflammatory cells and protein concentration) in the aqueous humor (AH) over 56 days. For in vivo drug delivery, implants were loaded with triamcinolone acetonide (TA). TA was extracted from biological samples and concentrations measured by HPLC.

**Results** Best results were obtained using PMM2.1.2 polymer (Mw >100'000) and either oligomers of PMM2.1.2 or ethoxylated derivatives of stearic acid (Simulsol®). The total clinical score of implanted eyes was ≤ 1 at d1, d2 and d3 and zero on following days. Over 56 days, AH showed <1mg/ml of proteins and <20 inflammatory cells / microl., values largely below levels characteristic of inflammation. Therapeutic concentrations of TA were detected in the vitreous (up to 935 ng/100 microl) and sclera (up to 510 ng/mg) up to 5 weeks. This is an advantage over subtenon injection of Kenacort®, where concentrations of TA increase till d11, then decrease and disappear at d21.

**Conclusion** 1. Scleral implants were successfully made with PMM2.1.2, a biodegradable polymer. 2. A good biocompatibility was demonstrated in rabbits. 3. Implants were able to prolong TA release when compared with injection of Kenacort® suspension. 4. The sclera plays a role of reservoir for lipophilic drugs such as TA.

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## Expression of MMP´s and TIMP by biodegradable biopolymer implants in rat eye.

*RÖNKKÖ S (1), OKSALA E (1), KAARNIRANTA K (1), SALMINEN A (2), TERÄSVIRTA M (1), UUSITALO H (1)*
*(1) Ophthalmology, Kuopio*
*(2) Neuroscience and Neurology, Kuopio*

**Purpose** Different undegradable biomaterials have widely been used in ophthalmology for many decades. During the last years studies around of degradable biomaterials have been increased its popularity. In this study, Inion GTR membrane (BM I), a blend of 85:15 PLGA and 70:30 poly(L-lactide-co-1,3-trimethylene carbonate) copolymers in a molar ratio of 70:30, PLGA (BM II; poly DL-lactide-coglycolide, 50:50), PLGA (BM III; 85:15) biomaterials were used in rat eye to unravel theirs biocompatibility.

**Methods** The BM I-BM III biomaterials were put into Wistar rat subconjuctival space for 2 weeks, 1 and 3 months. Moreover, BM I was followed up to 12 months. Matrix metalloproteinases (MMPs; MMP-2,-3 and -9) and tissue inhibitor (TIMP-2) were localized by immunohistochemistry.

**Results** The BM II and BM III showed initiation of degradative process after two weeks, and were not detected in samples collected after 6 or 12 months. However, the BM I was localized in the tissue up to 12 months. Immunohistochemical stainings of the MMPs and TIMP-2 were specifically associated with fibrous capsule formation. There were no significant differences in the expression levels of MMPs and TIMP-2 between biomaterials and in general, expression levels were rather constant at different time points. Furthermore, MMP-3 was found to slightly predominate in all samples.

**Conclusion** This study reveals that no clear biocompatibility changes between used biomaterials were observed, when reflected to MMPs and TIMP-2 immunihistochemical levels. In addition, the biomaterials showed different half-life in the rat eye. Our findings may help to develop new biomaterial application for improving results of eye surgery.

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## Intravitreal toxicity of Acular and Flurbiprofen

*MUNOZ MORALES A, PEYMAN GA, KIVILCIM M*
*Tulane University Health Sciences Center, New Orleans*

**Purpose** To determine the retinal toxicity of intravitreal injections of Acular and Flurbiprofen.

**Methods** Thirty-two New Zealand albino rabbits were divided into 8 groups (n=4 each) to evaluate four doses of intravitreally injected Acular: 125 microg, 250 microg, 500 microg and 1 mg, and the same four doses of intravitreally injected Flurbiprofen. One eye in each animal was used for the study dose while eight fellow eyes were injected with buffered sterile saline as the control group. All of the animals were examined using indirect ophthalmoscopy and slit-lamp biomicroscopy before and after intravitreal injection and at days 1, 7 and 14. An ERG was performed on all animals prior to intravitreal injection and 14 days after injection. On day 14 the animals were euthanized. Histological preparations of enucleated eyes were examined with light microscopy for retinal toxicity.

**Results** Clinical exam, histological evaluation and ERG results of the Acular and Flurbiprofen groups demonstrated no signs of retinal toxicity in all the groups .

**Conclusion** High doses up to 1 mg of Acular and up to 1 mg of Flurbiprofen injected intravitreally did not cause retinal toxicity. Intravitreal doses of up to 1 mg of Acular and Flurbiprofen may provide a new alternative for the treatment of ocular inflammatory diseases.

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## Different Outwardly Recifying Potassium Currents in the Mouse Retinal Rigment Epithelium

*WIMMERS S, KARL MO, STRAUSS O*
*University Medical Center Hamburg-Eppendorf / Department of Ophthalmology, Hamburg*

**Purpose** The retinal pigment epithelium (RPE) supports the normal function of the photoreceptors. By transporting ions, metabolites, and water it helps to maintain an appropriate microinvironment for the function photoreceptors. Large potassium conductances in the RPE are directly and indirectly involved in these transport mechanisms.

**Methods** Cultured mouse RPE cells were investigated by means of the patch–clamp technique.

**Results** Mouse RPE expresses two distinct outwardly rectifying potassium conductances, one of them activates at potentials positive to –10 mV and shows no inactivation. This current was sensitive to 10 nM agitoxin–2 (reduction to 30,5%) supporting the idea that it is carried by a potassium channel of the Kv1.x family. Additionally, we found an outwardly rectifying potassium current which activates at potentials more positive to –60 mV with slow inactivation at very positive potentials. This current was half–maximally activated at –35 mV.

**Conclusion** The molecular correlate of these potassium conductances is not yet identified. Because of its negative activation threshold this channel is open at physiological potentials and may contribute to different aspects of the RPE transport physiology.

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## An animal model to study the relationship between blood pressure and intraocular pressure

*SAVY O (1, 2), ROMANET JP (1), NOEL C (1), MOUILLON MM (1), BOURDON L (2)*
*(1) Service d'Ophtalmologie CHU Grenoble, Grenoble*
*(2) Centre de Recherches du Service de Santé des Armées, Grenoble*

**Purpose** To determine the relationship between intraocular pressure (IOP) and arterial blood pressure (BP) in an animal model, during a short period.

**Methods** Blood pressures were measured with two indwelled catheters inserted in the left carotid artery and jugular vein and IOP were measured with catheter inserted through the cornea into the anterior chamber of both eyes of anesthetized pigs (n=7) under spontaneous ventilation.Plateaus of different levels of BP (about 40, 50… to 140 mm Hg) were achieved by infusion with a syringe-pump of either sodium nitroprusside or angiotensine II at sufficient dosage. After equilibration of both BP and IOP for each plateau, the compliance of the eye ball was estimated by the analysis of the change in IOP induced by direct injection of a known volume (3, 6, 9 and 12 mL) of based saline solution into the anterior chamber with a microdialysis syringe pump.

**Results** IOP oscillates synchronously to BP with an amplitude of 2.5 ± 1.5 mm Hg in the right eye and 1.0 ± 0.8 mm Hg in the left one (occluded vessels). A good linear relationship between IOP and BP is observed instantaneously ($r > 0.9$; $p<0.001$) as well as in average ($r > 0.85$; $p<0.001$), the dependence of IOP in BP being about 20 % which means that a variation of 20 mmHg in mean BP induced a variation of 4 mmHg in mean IOP. The compliance of the eyeball is very low since an injection of as few as 3 mL induces an increase of IOP as high as 1 mm Hg in average; it slightly varies with BP.

**Conclusion** In this experimental study, the relationship between IOP and BP is more important than what is usually reported. Three hypotheses are discussed: a variation of aqueous humor secretion, a variation of eyeball compliance and a choroidal pulse.

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## Endocannabinoid and palmitoylethanolamide levels in eyes with diabetic retinopathy or macular degeneration

*MATIAS I (1, 2), WANG J (2), SCHIANO MORIELLO A (1), NIEVES AL (2), VALENTI M (1), DI MARZO V (1), WOODWARD D (2)*
*(1) Istituto di Chimica Biomolecolare - Consiglio Nazionale delle Ricerche, Pozzuoli*
*(2) Department of Biological Sciences, Allergan Inc., Irvine*

**Purpose** Cannabinoids and endocannabinoids, have been reported to lower intraocular pressure. In glaucomatous human eyes, we previously demonstrated an significant reduction of 2-arachidonoylglycerol (2-AG) levels in the ciliary body suggesting that pathological alterations in endocannabinoid levels may have a role in this disease state. Then, we investigated the levels of the endocannabinoids (2-AG), anandamide (AEA), and palmitoylethanolamide (PEA) as an "entourage compound" in different ocular tissues from normal patients, patients with diabetic retinopathy (DR) or macular degeneration (MD).

**Methods** 2-AG, PEA, and AEA in ocular tissues were extracted in chloroform/methanol. Deuterated standards were added as internal standards. The endocannabinoids were separated and subjected to liquid chromatography-mass spectrometry.

**Results** In eyes with DR, significantly enhanced levels of AEA were found in the retina (1.8-fold), ciliary body (1.5-fold) and, to a lesser extent, cornea (1.3-fold). Surprisingly, 2-AG levels were significantly higher (3-fold) only in the iris, which is not involved in DR, whereas PEA levels only slightly increased (1.3-fold) in the ciliary body. In eyes with MD, significantly enhanced levels of AEA were found in the choroid (1.3-fold), ciliary body (1.4-fold) and cornea (1.4-fold), whereas in the retina only a trend towards increase (1.5-fold) was observed. No significant changes in the levels of 2-AG or PEA were detected in eyes with this disease.

**Conclusion** Despite its limitations, this study suggests that endocannabinoids may be involved in the control of eye function under physiological and pathological conditions.

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## In vivo intra-ocular delivery of oligonucleotides using photosensitive vectosomes

*VALAMANESH F (1, 2), NORMAND N (1), BEJJANI RA (1), BENEZRA D (3), COURTOIS Y (1), BEHAR-COHEN F (1, 2)*
*(1) INSERM U598, Paris*
*(2) Laboratoire d'Innovation Thérapeutique en Ophtalmologie, Fondation Ophtalmologique A. de Rothschild, Paris*
*(3) Hadassah Hebrew University Hospital, Jerusalem*

**Purpose** To evaluate the potential of VP22, a structural protein of the herpes simplex virus tegument, for the delivery of oligonucleotides(ODN) to rat eyes.

**Methods** The C-terminal half of VP22 was expressed in Escherichia Coli and purified in high yields. VP22 was mixed with fluorescent phosphorothioate ODN in PBS for the preparation of particles, known as vectosomes. The vectosomes were administered to Lewis rats by intravitreal injections. The eyes were illuminated transclerally the following day using either white light or a laser beam using the excitation wave-length of the fluorochrome used to label the oligonucleotides (532nm). Subsequent studies of the intraocular distribution of the ODN were carried out. Cryosectioned were fixed in 4% paraformaldehyde and observed by fluorescence microscopy.

**Results** Vectosomes are observed 24h after injection in the cornea, the ciliary body, all retinal layers and more specifically in ganglion cells, the inner nuclear layer, the outer nuclear layer and the retinal pigment epithelial (RPE) cells. Some vectosome are also localized in the choroid. In the non-illuminated eyes, the pattern of fluorescence was punctuate and confined to the cytoplasm of ocular cells. The illumination triggered a disruption of the particles and lead to a redistribution of the fluorescent ODN from the cell cytoplasm to the cell nuclei. We observed no inflammatory response following two intravitreal injections with a three-week interval in between.

**Conclusion** Efficient and controllable site-specific delivery of therapeutic nucleic acids to the eye may be achieved using VP22 protein by simple and direct illumination with the lasers currently used in ophthalmology.

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## Curcumin treatment protects rat retinal neurons against excitotoxicity: effect on N-methyl-D-aspartate -induced intracellular Ca2+increase

*SCALZO G (1), MATTEUCCI A (2), FRANK C (3), DOMENICI MR (3), PARADISI S (2), SCORCIA V (1), BRUZZICHESSI D (1), BALDUZZI M (4), MALCHIODI-ALBEDI F (2), SCORCIA G (1)*
*(1) Eye Clinic, University of Catanzaro "Magna Graecia", Catanzaro*
*(2) Dep Cell Biology and Neuroscience, ISS, Rome*
*(3) Dep Drug Research and Evaluation, ISS, Rome*
*(4) UTS Biotechnologies, Protection of Health and Ecosystems, ENEA,, Rome*

**Purpose** Excitotoxicity is involved in the pathogenesis of neurodegenerative retinal conditions,such as glaucoma and diabetic retinopathy.Since the N-methyl-D-aspartate (NMDA)subtype of glutamate receptors seems to be particularly involved in the pathologic activation leading to neurodegeneration,several therapeutic strategies have been developed to antagonize NMDA receptor-related toxicity. Unfortunately, antagonists which completely block NMDA receptors cause numerous side effects, such as memory impairment, psychotomimetic effects, ataxia. Curcumin, an extract from the plant Curcuma longa with well-known antioxidant and anti-inflammatory activities, has been found to protect in a rat experimental model of cerebral ischemia/reperfusion, where excitotoxicity plays a major role.

**Methods** This observation prompted us to test curcumin as protective agent against excitotoxicity in rat retinal mixed cultures

**Results** A 24 h-treatment with curcumin reduced NMDA-mediated excitotoxic cell damage,estimated as decrease of cell viability (MTT) and increase in apoptosis (TUNEL).By double immunocytochemical labelling,we found that NMDA pro-apoptotic effect mostly affected GABA-ergic neurons (amacrine and horizontal cells),as compared to rhodopsin-positive photoreceptors,and was reverted by curcumin treatment.The protection was associated with decrease of NMDA receptor-mediated Ca2+ rise and reduction in the level of phosphorylated NR1 subunit of NMDA receptor.

**Conclusion** These results enlighten a new pharmacological action of the plant extract,possibly mediated by a modulation of NMDA receptor activity.

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## How much doxycycline reaches the ocular surface in patients undergoing therapy for ocular surface disease?

*KHAN-LIM D (1), SMITH VA (2)*
*(1) Ophthalmology, Southampton*
*(2) Ophthalmology, Bristol*

**Purpose** Oral doxycycline (α-6 Deoxy-5-hydroxytetracycline) is an established and effective treatment for a number of ocular surface disorders. Its therapeutic value has been ascribed to a concentration dependent ability to inhibit MMP activity and also both MMP and interleukin-1α synthesis. To what extent this drug reaches the tearfilm is currently unknown. The concentration of systemically administered doxycycline and MMP levels in the tears of patients with ocular surface diseases was therefore determined.

**Methods** Tear and blood samples were obtained from patients with ocular surface disease before and during doxycycline treatment (100mg per day). Healthy volunteers not undergoing treatment provided control samples. Tear samples were analysed for MMP activity by zymography. The doxycycline present in tear and blood samples was extracted with n-butanol and quantified spectrophotometrically.

**Results** The MMPs (of Mr 116,000 and MMP-9) present in the tears of patients with ocular surface diseases could not be detected after commencing systemic doxycycline treatment. Despite this doxycycline was not detected in their tears and the estimated concentration of doxycycline in their blood was around 4.5uM.

**Conclusion** The absence of doxycycline in the tears of patients treated for ocular surface disease suggests that its effect does not involve local modulation of the inflamed ocular surface. Since tear MMPs are the product of neutrophils, it is likely that the effectiveness of doxycycline therapy for ocular surface disease is related to the systemic targeting and inactivation of neutrophils.

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## Effects of Cyclooxygenase inhibitors on apoptotic retinal ganglion cells

*BRUST AK (1), ULBRICH H (2), PFEIFFER N (1), GRUS F (1)*
*(1) Ophthalmology, Mainz*
*(2) Institute of Pharmacy, Mainz*

**Purpose** Glaucomatous optic neuropathy is characterized by a loss of retinal ganglion cells which is associated with the decrease of visual function.Neuroprotective agents could prevent the remaining neurons from apoptotic cell death.Previous studies have shown that induction of the Cyclooxygenase-2 signaling cascade may sensitize neocortical and amnion-derived cells to apoptotic death. In our study we investigated the neuroprotective effect of different drugs, in particular Cyclooxygenase-1/Cyclooxygenase-2- (COX-1/COX-2) and selective COX-2- inhibitors on apoptotic retinal ganglion cells (R28).

**Methods** To study the effect of the substances we induced apoptosis in a retinal ganglion cell line (R28) by serum deprivation for 48 hours. This treatment caused a reduction of cell viability which was measured by the colorimetric WST-1 test (Roche Diagnostics, Mannheim, Germany). To test a possible relationship between induction of COX-2 expression and apoptotic cell death elicited by serum deprivation the cells were treated in the presence or absence of both COX-1/COX-2 inhibitors and selective COX-2 inhibitors. The expression of COX-1 and COX-2 in these cells was confirmed by western blot analysis.

**Results** We demonstrated that different COX-2 inhibitors prevent retinal ganglion cells from apoptosis in a dose dependent manner. COX-2 inhibitors seem to be more potent than inhibitors of COX-1.

**Conclusion** Treatment of apoptotic retinal ganglion cells with COX-2 selective inhibitors could increase the amount of surviving retinal ganglion cells in a dose dependent manner and could provide new opportunities for the neuroprotective treatment of apoptosis mediated diseases such as glaucoma.

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## Effects on the ciliary processes after experimental vitreous replacement with Perfluorohexyloctane

*LAKE S (1), DIAFAS SG (1), KARAMPATAKIS VE (2), KOMNENOU AT (3), NTAMPOS KS (2), MANTHOS AK (4)*
*(1) Ophthalmology, Aristotle University of Thessaloniki, Thessaloniki*
*(2) Laboratory of Experimental Ophthalmology, Aristotle University of Thessaloniki, Thessaloniki*
*(3) Clinic of Surgery, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki*
*(4) Laboratory of Histology-Embryology, Aristotle University of Thessaloniki, Thessaloniki*

**Purpose** To evaluate the effects of perfluorohexyloctane (F6H8) on the ciliary processes in rabbit eyes.

**Methods** 12 eyes of pigmented rabbits underwent unilaterally mechanical pars plana vitrectomy and vitreous replacement with F6H8 in 9 eyes or BSS in 3 eyes. 4 animals, 3 of the F6H8 and 1 of the BSS group, were sacrificed each time as follows: 24 h, 7 d, 36 d postop. After enucleation, each ciliary body was divided into superior (2) and inferior (2) portions and processed for transmission electron microscopy (TEM). Specimens of the inferior ciliary body in contact with the F6H8 were compared with specimens of the superior ciliary body.

**Results** In the posterior chamber, dispersion of F6H8 was observed early from the 6th day and was dense in the 36 days group. Some eyes depicted few whitish precipitates in the vitreous.On TEM all the control eyes (BSS) were free of pathological changes. In the F6H8 group, the ciliary epithelial cells were normal on the 1st day. On the 7th day vacuolar changes and mild widening of the intracellular spaces were rarely observed. On the 36th day the main findings were vacuolar and myelinoid alterations in the ciliary epithelial cells. The alterations were more prominent in the inferior ciliary processes. Histological studies revealed no preretinal membrane formation, no edema of the mitochondria, no nuclear drop-outs by the ciliary epithelium cells and the stroma of the ciliary body was normal.

**Conclusion** The most important clinical complication is the dispersion of the F6H8 liquid into droplets. TEM showed that the alterations of the ciliary processes were minor, non-specific and mild. Our findings suggest that F6H8 was tolerated by the ciliary body for 36 days.

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## Cessation of Systane® Lubricant Eye Drops and Return of Signs and Symptoms of Dry Eye

*PRZYDRYGA J (1), COHEN S (2), CHRISTENSEN M (1)*
*(1) Alcon Labs, Fort Worth, TX*
*(2) Private Practice, Scottsdale, AZ*

**Purpose** To determine the length of time after removal of an effective dry eye treatment before patients returned to a state of significant dry eye.

**Methods** 20 successful users of Systane were enrolled at one site in an open label non-controlled clinical study of 5 weeks duration. Average pre-study Systane use was 2.1/day. Corneal staining (sodium fluorescein, NEI grid; sum of 5 areas; 0-3 scale/area), conjunctival staining (Lissamine Green, NEI grid; sum of 6 areas, 0-3 scale/area) and symptom frequency were obtained at each visit. The Schein questionnaire was used to obtain symptom frequency (1 = none; 4 = all of the time for Burning, Dryness, Grittiness, Redness, Crusty Lashes and Stuck Eyelids). Patients were told to discontinue Systane use and return for reevaluation on a weekly basis. Patients could return to treatment only after evaluation by the investigator.

**Results** Two patients exited after one week off treatment and five made it to 5 weeks (Average time off treatment = 3.5 weeks). Mean staining at entry was 2.03 for cornea and 2.08 for conjunctival. A comparison between entry/exit visits showed a significant change from baseline increase in both mean sum of corneal staining (3.90; $p < 0.0001$) and conjunctival staining (3.43; $p < 0.0001$). Symptom frequency also showed significant increases from baseline for Burning ($p=0.0004$), Dryness ($p<0.0001$), Grittiness ($p=0.0005$) and Redness ($p=0.0141$) with a trend for Crusty (0.0583). Stuck eyelids did not show a significant difference ($p=0.2845$). A composite of all symptoms did demonstrate a highly statistically significant ($p<0.0001$) change from baseline.

**Conclusion** Previous studies have demonstrated Systane's efficacy when compared to a marketed control. This study showed that upon removal of treatment with Systane, patient's signs and symptoms return on average, 3.5 weeks off treatment. This study was sponsored and conducted by Alcon Research Ltd, Fort Worth, TX USA.

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## The effect of smoking on foveal function

*NILSSON M, HENRICSSON A, MARTIN L*
*Clinical Neuroscience, Stockholm*

**Purpose** To evaluate foveal function in smoking and non-smoking subjects using the RFT technique.

**Methods** The Rarebit Fovea Test (RFT) (Malmer & Martin 2005), relies on the perception of very small (< 0.5 MAR) bright stimuli. The test area covers the fovea and its immediate surrounding (approximately 3°). The results are expressed as hit rate i.e. percentages of dots seen. Right eyes from 17 healthy smokers (age 45.6±9.6) and 26 non-smokers (age in both groups 42.6±9.6) with of visual acuity (VA) ≥ 1.0 were examined using the RFT technique.

**Results** A statistically significant ($p <0.0001$) lower mean hit rate was observed in the smoking group compared to the non-smoking group. There was no significant difference in VA between groups (1.4±0.3) and no correlation was found between hit rate and VA in either group.

**Conclusion** The results in the current study indicate that smoking have an effect on the foveal function that can be revealed using the Rarebit Fovea Test.

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## A Pilot Study Comparing Sub-conjunctival Triamcinolone Injection with Topical Steroid in Controlling Post-Cataract Surgery Inflammation

*CHIAM P, CHAN J, PATEL P, KASABY H*
*Eye Department, Southend*

**Purpose** To determine whether sub-conjunctival triamcinolone acetonide injection treats post-cataract surgery inflammation as well as topical dexamethasone 0.1%.

**Methods** 50 eyes were prospectively randomized to receive either sub-conjunctival 40mg triamcinolone acetonide injection at the end of cataract surgery (without post-operative topical steroid) or steroid (dexamethasone) and antibiotic (chloramphenicol) drops post-operatively. A single surgeon performed all surgery without complication. Patients were followed-up post-operatively at day 3, 7, 14 and 28. The patients and examiner were masked. The primary efficacy variables were anterior chamber cell and flare. Other variables measured were IOP, conjunctival erythema, best corrected visual acuity (BCVA) and subjective post-operative ocular pain.

**Results** There was no statistically significant difference between the two groups in terms of anterior chamber cell ($p>0.58$) and flare ($p>0.76$) at all postoperative days. The triamcinolone group has less conjunctival erythema at day 3 ($p<0.03$) and 7 ($p<0.04$) post-operatively. There was no statistically significant difference in the intraocular pressure or ocular pain at all post-operative days. No patients from either group were dropped from the study because of lack of anti-inflammatory response.

**Conclusion** Per-operative sub-conjunctival 40mg triamcinolone acetonide injection demonstrated no statistically significant difference compared with post-operative topical dexamethasone 0.1% in controlling post-cataract surgery inflammation.

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## Effects of physical exercise on intraocular pressure after the instillation of brimonidine tartrate eye drops

*KARAMPATAKIS VE (1), NATSIS K (2), DIAFAS SG (3), CHATZIBALIS T (1), NTAMPOS KS (1), LAKE S (3)*
*(1) Laboratory of Experimental Ophthalmology, Aristotle University of Thessaloniki, Thessaloniki*
*(2) Anatomy, Aristotle University of Thessaloniki, Thessaloniki*
*(3) Ophthalmology, Aristotle University of Thessaloniki, Thessaloniki*

**Purpose** To evaluate the influence of physical aerobic exercise on intraocular pressure (IOP), after the instillation of a selective a2 – adrenergic receptor agonist (brimonidine tartrate 0.2%).

**Methods** The IOP of both eyes was measured by applanation tonometry in 15 healthy individuals. Brimonidine tartrate 0.2% eye drops were then instilled in the right eye of the individuals and 2 hours later the IOP was measured again. Thereafter, the individuals performed a physical exercise on a bicycle ergometer for about 10 minutes at 60-80 Watts. IOP was measured again within 5 minutes post-exercise.

**Results** The mean IOP of the right eyes before the instillation of brimonidine was (mean ± SD) 14.66 ± 2.38 mmHg and two hours after 10.26 ± 2.34 mmHg (statistically significant difference, $P < 0.001$). After the completion of the physical exercise the mean IOP of the right eyes was 7.4 ± 2.02 mmHg (statistically significant difference, $P < 0.001$). As regards the left eyes, the mean IOP before the a2 – agonist instillation on the fellow eye was 14.06 ± 2.08mmHg and two hours after 13.06 ± 2.73mmHg ($P = 0.01$). Post-exercise, mean IOP of the left eyes was 10.4 ± 2.55 mmHg (statistically significant difference, $P < 0.001$). The mean IOP lowering effect of the exercise was 2.86 ± 2.09 mmHg for the right eyes and 2.66 ± 1.67 mmHg for the left eyes (no statistically significant difference between the treated and untreated eyes, $P = 0.775$).

**Conclusion** The data indicate that IOP decreases after a physical aerobic exercise regardless of the instillation of the selective a2 – adrenergic receptor agonist.

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## Intercellular communication in retinal endothelial is downregulated in response to high glucose by a proteasome dependent mechanism

*PEREIRA P, GIRAO H, FERNANDES R*
*Centre of Ophthalmology, Faculty of Medicine, University of Coimbra, Coimbra*

**Purpose** Disruption of Intercellular communication through gap junctions (GJIC) is associated with endothelial dysfunction characteristic of diabetic retinopathy. This study is designed to investigate the mechanisms whereby high glucose enhances degradation of connexin 43 (Cx43) by a proteasome dependent pathway, contributing to decrease GJIC under high glucose.

**Methods** Bovine retinal endothelial cells (BREC) were exposed to high glucose (25 mM) in the presence or absence of proteasome inhibitor MG132. The levels of Cx43 were assessed by western blot. The contribution of the proteasome for the degradation of Cx43 in high glucose was evaluated by metabolic labelling in the presence of proteasome inhibitors. Subcellular distribution of Cx43 was determined by immunofluorescence confocal microscopy and further confirmed by biotinylation of cell surface proteins. (GJIC) was evaluated by transfer of the dye Lucifer yellow.

**Results** The levels of Cx43 in BREC exposed to high glucose decreased about 50% as compared to controls (5.5 mM glucose), whereas the half-life of the protein decreased from 2.3 h to 1.9 h. Moreover GJIC decreased about 40% in response to high glucose. Proteasome inhibitors prevented the loss of Cx43 induced by high glucose by decreasing Cx43 turnover and reverted the decrease in GJIC induced by high glucose. The amount of phosphorylated Cx43 increased both in high glucose and following proteasome inhibition.

**Conclusion** Degradation of Cx43 by a proteasome dependent mechanism is likely to be a novel regulator of intracellular communication under high glucose. Dowregulation of intercellular communication is likely to be of relevance in endothelial dysfunction in diabetic retinopathy.

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### Ability of the OCT to detect patients with preperimetric glaucoma

*LALIENA SANTAMARIA JL (1), PARDINAS BARON N (1), GARCIA ABELLAN V (1), GARCIA FEIJOO J (2), SANCHEZ-CANO A (1), PEREZ OLIVAN S (1)*
*(1) Hospital Universitario Miguel Servet, Zaragoza*
*(2) Instituo De Investigaciones Oftalmológicas Ramón Castroviejo De La Universidad Complutense De Madrid, Madrid*

**Purpose** To study the ability of OCT for the detection of patients with preperimetric glaucoma , that is to say, the eyes with structural alteration in the retinal nerve fiber layer or progression of the damage in optic nerve head, but without perimetric defects.

**Methods** 250 eyes of 250 patients, distributed of the following form were evaluated: group control (49 eyes), group suspects (150 eyes) and to incipiente-moderate group glaucoma (51 eyes). All patients underwent standard automated perimetry, photos of fiber layer, photos of optic nerve head andquantitative exploration with OCT

**Results** The analysis by means of OCT showed that several measures was able to discriminate between the group glaucoma and the rest (average thickness, superior, superior maximum and inferior maximum) but no between the group suspects and the control. The internal analysis of the group suspects by means of curves ROC and areas under the curve (method of Hanley and McNeil) there demonstrated greater diagnostic yield of the measurements inferior maximum thickness and average thickness to label the eyes with a preperimetric glaucoma.

**Conclusion** The parameters of OCT with greater diagnostic yield for the detection of preperimetric glaucoma were the maximum inferior thickness and the average thickness.This work has been financed by the scholarship RTIC-C n º C03/13.

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### Quantification of neuroretinal rim loss using digital planimetry in long-term follow-up of normals and patients with ocular hypertension

*LAEMMER R, SCHROEDER S, JUENEMANN AG, VIESTENZ A, MARDIN CY*
*Department of Ophthalmology, Erlangen*

**Purpose** The purpose of this study was to investigate if digital planimetry is appropriate for quantification of neuroretinal rim loss in patients with ocular hypertension and if there is an age related neuroretinal rim loss in normals.

**Methods** Forty-two normals, 60 patients with ocular hypertension without progression and 14 patients with hypertension and conversion to early glaucoma from Erlanger Glaukomregister were included in the study. Patients and normals received complete ophthalmologic examination including detailed diagnos-tic concerning glaucoma. Optic disc images from baseline and after 5 or 10 years follow up were used for digital planimetry. Optic disc area and cup area were measured using commercial Software: Soft Imaging System analysisTM. The investigator was masked for diagnosis and time point of examination.

**Results** Mean neuroretinal rim loss was 0,27% per year in normals, 0,52% per year in patients with ocular hypertension without progression and 1,17% per year with ocular hypertension and conversion.

**Conclusion** Even in normals we detected a low neuroretinal rim loss during 10-year follow up with digital planimetry. In patients with ocular hypertension without progression neuroretinal rim loss was as twice as in normals. Mean neuroretinal rim loss of less than a few percent can be detected by digital planimetry that is even not visible to the trained observer.Supported by Deutsche Forschungsgemeinschaft SFB 539 "Glaukome und Pseudoexfoliationssyndrom"

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### Comparison of two cornea compensation methods in scanning laser polarimetry on eyes with atypical polarisation pattern

*TÓTH M (1), VARGHA P (2), HOLLÓ G (1)*
*(1) Department of Ophthalmology, Semmelweis University, Budapest*
*(2) Biometry Unit, Semmelweis University, Budapest*

**Purpose** To investigate the potential advantage of a recently developed cornea compensation algorithm (ECC) over to the present compensation method (VCC) in the analysis of scanning laser polarimetric (SLP) images with atypical retardation pattern (ARP).

**Methods** SLP-VCC images with ARP (typical scan score, TSS<80) of one eye of each of 27 glaucoma patients and 19 healthy subjects were compared to the corresponding SLP-ECC images obtained at the same session. For statistical analysis the Wilcoxon signed rank test and the paired t-test were used. P values of less than 0.05 were considered statistically significant.

**Results** ARP was present in 10.4 % of the normals and 15.5 % of the glaucoma patients imaged with SLP-VCC over nine months. In both groups TSS was higher for ECC than for VCC ($p<0.001$). In glaucoma TSNIT, superior and inferior average thickness values were significantly lower, and TSNIT standard deviation was significantly higher with ECC than with VCC ($p<0.001$for each comparison). In the normal group the nerve fibre indicator (NFI) was lower with ECC than with VCC ($p=0.007$). TSNIT average was smaller and TSNIT standard deviation was higher with ECC ($p<0.001$). Superior and inferior average thickness did not differ between VCC and ECC in the normal group.

**Conclusion** The new ECC software substantially improves polarimetric image analysis both on normal and glaucomatous eyes showing ARP, and therefore it may have clinical significance in glaucoma detection in the future.

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### Morphometric parameters of scanning laser tomograph (HRT-II) and scanning laser polarimetry (GDx-VCC) to discriminate between normal and glaucomatous eyes

*MILANO G (1, 2), SERRA LI (1, 2), MACERI C (1, 2), TISO C (1, 2), SCATASSI I (1, 2)*
*(1) Clinica Oculistica dell'Università, Pavia*
*(2) I.R.C.C.S. Policlinico San Matteo, Pavia*

**Purpose** To evaluate the ability of morphometric parameters provided by scanning laser tomography and scanning laser polarimetry in the detection of normal and pathologic eyes.

**Methods** 97 consecutive patients referring to the Glaucoma service of the University Eye Clinic of Pavia, were submitted to a complete ophthalmic examination including applanation tonometry, automated visual field testing (VF), confocal scanning laser ophthalmoscopy of the optic nerve head (ONH) with HRT-II and confocal scanning laser polarimetry of the retinal nerve fiber layer (RNFL) with GDx-VCC. All patients were divided into normal, suspect and glaucomatous subjects on the basis of automated perimetry and tonometry outcomes. Among the parameters provided by HRT-II: Cup Shape Measure (CSM), Cup Disk Ratio (CDR), Cup Area (CA), Rim Area (RA), mean RNFL thickness (MRNFLt) and RNFL cross sectional area (RNFLcsa) were taken into accountg. Amang the parameters provided by GDx: TSNIT average, TSNIT standard deviation and Nerve Fiber Indicator (NFI) were considered. The association of structure, as measured by GDx or HRT parameters, with clinical diagnosis was assessed by means of logistic models.

**Results** The association of morphometric parameters with a diagnosis of glaucoma was rather good for both HRT and GDx, but only GDx parameters have a significative association with normal subjects. Among the morphometric parameters given by GDx, NFI provides the best association with clinical diagnosis.

**Conclusion** Both HRT and GDx provide a good discrimination of glaucomatous patients. GDx can point out normal subjects too. NFI seems to be the most useful morphometric parameter in clinical practice.

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## A prospective long-term study about morphological and functional test for the early diagnostic of glaucoma

*GARCIA ABELLAN V (1), PARDINAS BARON N (1), LALIENA SANTAMARIA JL (1), FERNANDEZ LARRIPA S (1), PEREZ OLIVAN S (1), PABLO JULVEZ L (1), MARTINEZ DE LA CASA JM (2)*
*(1) Hospital Miguel Servet, Zaragoza*
*(2) Hospital Clinico Universitario, Madrid*

**Purpose** To evaluate the results of a long-term monitoring of a group of subjects according to differents test: standardized automated perimetry, color stereoscopic photography of the optic disc and red-free photography of the retinal never fiber layer and to compare the efficiency of these test to detect the early glaucoma damage.

**Methods** We realized an ophtalmological basic exploration beside the other tests mentioned previously. The patients who cumplied our criteria of inclusion (251 patients) were divided in three groups: normal subjects (50), suspect of glaucoma (163) and patients with early glaucoma (38). We follow the patients and all the tests were realized annually. The results were analyzed with several statistical test: ANOVA, ROC area, Pearson.

**Results** There were significant statistically differences in the evaluation of the never fiber layer between normal subjects and suspects, also between normal and glaucoma group along the study. At first evaluation were differences between suspect and glaucoma early damaged eyes. There were also differences in the perimetry between the control group and the glaucoma group along the study. The photography of the never fiber layer has more capacity to discriminate among groups than the presence of PIO>25 or a relation cup/disc > 0.7 ($p<0.05$).

**Conclusion** The cost-effectiveness of the photography of the never fiber layer is very important and some patients can take advantage of an early diagnostic and treatment. This work has been financed by RTIC/CnºC03/13

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## Correlation between retinal nerve fiber layer measurements performed by means of optical coherence tomography and scanning laser polarimeter GDx VCC

*FERRERAS A (1), POLO V (1), LARROSA JM (1), PAJARIN AB (2), PUEYO V (1), HONRUBIA FM (1)*
*(1) Ophthalmology, Zaragoza*
*(2) Primary Care, Zaragoza*

**Purpose** To correlate retinal the nerve fiber layer (RNFL) thickness and the parameters measured by optical coherence tomography (OCT) with the parameters obtained with the laser polarimeter GDx VCC in normal, ocular hypertensive and glaucomatous eyes.

**Methods** A total of 66 normal, 285 ocular hypertensive and 74 glaucomatous subjects were included in the study. These eyes were classified in diagnostic groups based on intraocular pressure, stereophotographs of the optic disc and Humphrey achromatic perimetry. Pearson correlations between RNFL thickness parameters of both devices were calculated.

**Results** In the total group, significant correlations were found between all OCT parameters and most GDx parameters, except for the 9 hour-position, that did not show any significant correlation. The average thickness and the nerve fiber indicator (NFI) showed the strongest correlation (-0.742).Normal, hypertensive and glaucomatous eyes presented less significant correlations. Most parameters showed moderate correlations in the 3 groups, and the strongest correlations were found with the average thickness (OCT) and the NFI (GDx).

**Conclusion** The correlations found between RNFL parameters of both instruments support the usefulness of the OCT and the GDx to assess the structural damage in glaucoma.

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## Heidelberg retina tomography versus optical coherence tomography in the optic nerve head analysis: a correlation study

*FERRERAS A (1), LARROSA JM (1), POLO V (1), PAJARIN AB (2), MASANA C (1), HONRUBIA FM (1)*
*(1) Ophthalmology, Zaragoza*
*(2) Primary Care, Zaragoza*

**Purpose** To evaluate the correlations of the optic nerve head parameters measured with the Heidelberg retina tomography (HRT II) and the optical coherence tomograph (Stratus OCT 3000) in normal, ocular hypertensive and glaucomatous eyes.

**Methods** 425 eyes of 425 subjects were included in the study, and were classified in 66 normal, 285 ocular hypertensive and 74 glaucomatous subjects based on intraocular pressure, stereophotographs of the optic disc and Humphrey achromatic perimetry. Pearson correlations between global HRT II parameters and optic nerve head OCT parameters were calculated.

**Results** Significant correlations ($p<0.05$) were found for most parameters of both instruments in all the groups. The strongest correlations were observed for disc area and cup area in the total group (0.725 and 0.774, respectively) and in the 3 diagnostic groups. Glaucomatous eyes showed the strongest correlations: cup area and vertical cup/disc area ratio (0.811 and 0.805, respectively). Normal group presented the less strong correlations (disc area: 0.516 and horizontal cup/disc area ratio: 0.505).

**Conclusion** Optic nerve head evaluation by means of HRT II and OCT 3000 shows similar results in glaucoma diagnosis.

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## Clinical ability of Heidelberg Retinal Tomograph II to diagnose early glaucoma

*PARDINAS BARON N, LALIENA SANTAMARíA JL, GARCIA ABELLAN V, PABLO JULVEZ L, HONRUBIA FM*
*Oftalmologia (Hospital Miguel Servet), Zaragoza*

**Purpose** To study the ability of HRT II to detect glaucomatous change in a sample divided into normal subjects; glaucomatous suspect and early glaucoma.

**Methods** 250 eyes are taken from 250 patients. 49 normal eyes;150 glaucomatous suspect (19 of them preperimetric glaucoma: normal automated perimetry but changes in optic disc in exploration and stereophotograph or retinal nerve fiber layer defect with SLO ) and 51 early glaucoma.It is necessary changes in the automated perimetry to diagnose glaucoma; considering itself like gold standard.

**Results** Statistically significant differences in several HRT II parameters, between normal and glaucomatous subjects, exist. Batija function showed the best capacity to differentiate between early glaucoma and glaucomatous suspect; as just glaucoma preperimetric from other glaucomatous suspect ( with the bigest area under ROC curves)

**Conclusion** HRT II is able to differentiate between glaucomatous and normal eyes; nevertheless its useful is limited when glaucomatous suspect eyes and/or preperimetric glaucoma are undergone to study.

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## Retinal nerve fiber layer assessment by means of Optical Coherence Tomography in glaucoma diagnosis

*MAYORAL F, POLO V, FERRERAS A, LARROSA JM, LOPEZ MJ, PUEYO V, SANCHEZ-CANO A, HONRUBIA FM*
*Servicio de Oftalmología - Hospital Miguel Servet, Zaragoza*

**Purpose** To assess the retinal nerve fiber layer thickness measured by optical coherente tomograpy (OCT) in normal subjects, ocular hypertensives with normal short-wavelength automated perimetry (SWAP), ocular hypertensives with abnormal SWAP and glaucomatous eyes.

**Methods** 98 normal subjects, 156 ocular hypertensives with normal SWAP, 21 ocular hypertensives with abnormal SWAP and 66 glaucomatous patients were included in the study. The groups were defined based on intraocular pressure, standard automated perimetry and SWAP performances and optic disc stereophotograph evaluation. RNFL assessment was performed by means of OCT (OCT 3000, Humphrey Zeiss instruments). Average and segmental (quadrants and clock hours) RNFL thickness values were compared among all the groups.

**Results** In the glaucoma group the RNFL thickness was thinner in all the parameters compared to the normal group. There were statistically significative differences between the groups of glaucoma and ocular hypertension with altered SWAP in global RNFL thickness, all quadrants (except temporal quadrant) and all hour-positions (except positions 9, 10 and 11). Also, the differences between the normal group and ocular hypertension with altered SWAP were only significative at the 11-hour position.

**Conclusion** RNFL assessment by means of OCT showed significative differences between the groups of the study. The use of OCT may improve our diagnostic ability to detect glaucoma in clinical practice

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## Analysis of optic disc in normal subjects, glaucoma suspects and glaucoma patients with Confocal Scanning Laser (HRT II)

*ALIAS E, LARROSA JM, FERRERAS A, POLO V, LOPEZ MJ, BORQUE E, MAYORAL F, HONRUBIA FM*
*Servicio Oftalmología - Hospital Miguel Servet, Zaragoza*

**Purpose** To assess the diagnostic ability of confocal scanning laser HRT II to detect glaucomatous optic damage in normal subjects, glaucoma suspects (short-wavelength automated perimetry altered) and patients with glaucoma.

**Methods** A total of 101 normal eyes, 46 glaucoma suspects, and 102 glaucomatous eyes (standard automated perimetry altered) were included in study. Every patient underwent complete ophthalmic examination. Statistical differences among the 3 groups of study were tested by means of ANOVA analysis. Also, ROC curves were plotted for the different optic disc parameters to compare their diagnostic value and sensitivity and specificity balance.

**Results** There were statistically significant differences between normal and glaucomatous eyes in most optic nerve head parameters ($p<0.05$). Glaucoma suspects (ocular hypertensives with altered SWAP) showed a pronounced overlap of their optic nerve head parameters with respect to control and glaucoma groups. The parameters that best discriminate the presence of glaucomatous damage at a fixed specificity of 90% were rim area (specificity 63.7%), cup-to-disc area ratio (specificity 59.8%) and vertical cup-to-disc ratio (specificity 60.8%). In the group of glaucoma suspects the most discriminant parameters (at a fixed specificity of 90%) were rim volume (sensitivity 50%), cup shape measure (sensitivity 52.2%) and vertical cup-to-disc ratio (sensitivity 52.2%).

**Conclusion** The use of HRT II may be useful to detect the presence of glaucomatous damage at early stages of the disease despite the overlap of results between normal subjects, glaucoma suspects and glaucoma patients.

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## Correlation between perimetric indices of conventional automated perimetry and structural parameters obtained by means of OCT, HRT and GDx

*LOPEZ PEÑA MJ, LARROSA JM, POLO V, FERRERAS A, PUEYO V, BORQUE E, ALIAS E, HONRUBIA FM*
*Servicio Oftalmología - Hospital Miguel Servet, Zaragoza*

**Purpose** To determine the correlations between structural parameters of the optic disc and retinal nerve fiber layer obtained by means of ocular coherence tomography, Heidelberg Retina Tomograph and polarimetry with the perimetric indices of standard automated perimetry.

**Methods** A total of 66 normal subjects and 74 glaucomatous patients were included. Subject eyes were classified into the groups based on IOP and standard automated perimetry. Pearson's correlation coefficients were elaborated between structural parameters of optic disc and RNFL and the global perimetric indices.A $p<0.05$ was considered to be statistically significative

**Results** Significative correlations were found in the glaucoma group.The GDx-VCC parameters showed significative correlations with MD and with CPSD. These correlations in the glaucoma group were also significative for the most of the parameters of HRT with MD and CPSD. Cup shape measure showed statistically significant correlations with MD and CPSD. The OCT analysis also showed significant correlations in the glaucoma group between the perimetric indices and the structural parameters of optic nerve and RNFL. In the group of normal subjects the correlations found between structural and functional parameters were not significative for the GDx-VCC parameters. In this group of subjects the correlations of HRT and OCT parameters with perimetric indices were statistically significant only between the cup-to-disc ratios and the MD and CPSD parameters.

**Conclusion** The correlations found between structural parameters obtained by means of OCT, HRT and GDx and standard automated perimetry indices support their use in glaucoma diagnosis.

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## Diagnostic ability of analysis tools for detection of glaucoma with the Confocal Scanning Laser Tomograph (Heidelberg Retina Tomograph II)

*BORQUE E, FERRERAS A, POLO V, LARROSA JM, ALIAS E, MAYORAL F, SANCHEZ-CANO A, HONRUBIA FM*
*Servicio Oftalmología - Miguel Servet, Zaragoza*

**Purpose** To evaluate the performance of logistic regression formulas (LRGs) elaborated from our autoctone population (Zaragoza, Spain) by means of multivariate analysis of the topographic parameters obtained by Heidelberg Retina Tomograph to detect glaucomatous damage.

**Methods** 101 normal eyes, 247 ocular hypertensive eyes and 102 glaucomatous eyes were included. Subjects were classified into the 3 groups based on IOP and standard automated perimetry (SAP) performance. The receiver operating characteristic curves (ROC) and the area under curves (AUC) were performed to assess the diagnostic value of the 4 multivariate formulas elaborated from our autoctone population to discriminate the presence of glaucomatous damage.

**Results** There were significant differences between normal and glaucomatous eyes in all the LRGs ($p<0.05$). Ocular hypertensive eyes showed a pronounced overlap of the LRG results with respect to control and glaucoma groups. When ocular hypertensives were segregated into different subsets of eyes based on clinical evaluation of the optic nerve head or short-wavelength automatic perimetry performance, the LRG ability to discriminate the presence of structural glaucoma damage significantly improved. At a fixed specificity of 90% all the LRGs showed a sensitivity around 65% with AUCs greater than 0,84. A significant correlation was found between LRGs and the global indices of SAP.

**Conclusion** The use of HRT analytic tools improves the diagnostic ability of HRT to discriminate healthy subjects from glaucoma patients. The use of alternative tools based on normative databases of autoctone population also improves the value of these diagnostic tools.

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## Diagnostic value of the Stratus OCT Optical Coherence Tomograph, Heidelberg Retina Tomograph (HRT II) and GDx VCC Scanning Laser Polarimeter to detect structural damage in glaucomatous eyes

*PUEYO V, POLO V, LARROSA JM, FERRERAS A, MAYORAL F, BORQUE E, SANCHEZ-CANO A, HONRUBIA FM*
*Ophthalmology, Zaragoza*

**Purpose** To compare the diagnostic capacity to discriminate between normal and glaucomatous eyes of different optical imaging devices, Optical Coherence Tomography (OCT), Heidelberg Retina Tomograph (HRT), and GDx Nerve Fiber Analyzer (GDx VCC).

**Methods** A total of 66 normal subjects and 74 glaucomatous patients were included in the study. Subject eyes were classified into the diagnostic groups based on intraocular pressure and standard automated perimetry. Every patient underwent complete ophthalmic examination including GDx VCC, HRT II and Stratus OCT 3000 evaluation. The receiver operating characteristic curves (ROC) were plotted to obtain the diagnostic value (sensitivities at fixed specificities -85% and 95%-) and the area under curves (AUC) of the different structural parameters assessed by the optical imaging devices.

**Results** In glaucomatous eyes the best parameters from each device were the GDx VCC Nerve Fiber Index -NFI- (AUC=0,879), the OCT retinal nerve fiber layer global average thickness (AUC=0,945) and the HRT linear discriminant function FSM (AUC=0,899). No statistically significant differences were found between the AUCs for these parameters. Nevertheless, at a fixed specificity of 85% and 95% OCT showed better sensitivity than HRT and GDx ($p<0.05$).

**Conclusion** Several structural parameters measured by the optical imaging devices of this study are useful to discriminate glaucomatous damage with high diagnostic abilities. Nevertheless, the best OCT and HRT parameters showed higher sensitivities than the best GDx VCC parameters.

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## Dynamic Contour Tonometry after intraocular surgery

*KAMPPETER BA, JONAS JB*
*Department of Ophthalmology, Faculty for Clinical Medicine Mannheim, Ruprecht-Karls-University, Mannheim*

**Purpose** Postoperative intraocular pressure measurement is prone to error due to temporary alteration of bulbar and corneal properties, like corneal edema, incisions and gas tamponades. This study compares pressure readings of dynamic contour tonometry as a new method for intraocular pressure measurement with Goldmann applanation tonometry.

**Methods** The study included 31 eyes of 31 subjects (10 eyes after standard pars plana vitrectomy with C3F8 gas tamponade and 21 eyes after clear cornea incision phako emulsification. All measurements were performed one day postoperatively. The dynamic contour tonometric measurements were compared with Goldmann applanation tonometric values obtained at the same examination. Altogether, 93 dynamic contour tonometric measurements and 62 Goldmann tonometer measurements were analyzed. Additionally, corneal pachymetry was performed.

**Results** Mean dynamic contour tonometric measurements after cataract surgery (22.07 ± 7.21 mm Hg) did not show significant ($p=0.979$) differences when compared to mean applanation tonometric values (22.10 ± 8.39 mm Hg). Pressure measurements after surgery with gas tamponade was 21.52 ± 6.22 mm Hg for dynamic contour tonometry and 21.01 ± 7.63 mm Hg for applanation tonometry. Differences were not significant ($p=5.83$). No significant correlation between tonometric values and central corneal thickness were found.

**Conclusion** Dynamic contour tonometry and applanation tonometry pressure readings did not show significant differences in eyes after intraocular surgery. Compared to literature data, pressure values measured by dynamic contour tonometry seem to be slightly lower when compared to applanation tonometry.

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## Agreement between a new tonometer-ICARE- and Goldmann applanation

*TONINI MT, SAVY OS, RENARD ER, THURET G, CHIQUET CC, MOUILLON MM, ROMANET JPR*
*Ophthalmology, Grenoble*

**Purpose** The Goldmann applanation is the gold standard to measure intraocular pressure (IOP). Nonetheless several drawbacks remain: corneal contact exposes to an infectious risk, patient must usually be seated, measure requires a training, children and pusillanimous patients feel uncomfortable and any modification of the corneal surface can alter the results. Therefore new physical method of tonometry with less disadvantages needs to be evaluated. ICARE tonometer (TIOLAT, Finland) is based on the measurement of the speed of the rebound of a small harmless disposable probe thrown on to the cornea without topic anaesthesia. Aim: to determine the agreement between both methods.

**Methods** Three trained doctors of our glaucoma department performed 314 consecutives measures on 29 patients with both methods in a random order. Agreement between both tonometers was evaluated with the Bland and Altman method.

**Results** IOP with ICARE were significantly higher than with Goldmann applanation: 18+/-6 mmHg, (from 6 to 54) median 17 versus 16+/-6 mmHg (5 to 51), median 15, $p<0.001$. Bland Altman plots: a mean overestimation of 2 mmHg was described with ICARE and 95% of these measures were between -4 and +8 mmHg around Goldmann IOP. No variation of agreement in relation to the level of IOP was highlighted.

**Conclusion** The new ICARE tonometer slightly overestimated IOP but remains clinically relevant. Considering its advantages in case of patients suffering from dystrophic cornea, in certain early post operative period, in children, and lying patients, this user-friendly tonometer could be an alternative method to the applanation. The lower threshold of detection and absence of bias for high IOP make it usable for mass screening.

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## Comparison of the ICare® rebound tonometer with the Goldmann tonometer in a normal population

*FERNANDES P, DIAZ-REY JA, QUEIROS A, GONZALEZ-MEIJOME JM, JORJE J*
*Physics (Optometry), Braga*

**Purpose** The aim of this study was to evaluate the accuracy of measurement of intraocular pressure (IOP) using a new induction/impact rebound tonometer (ICare®) in comparison to the Goldmann applanation tonometer (AT).

**Methods** Forty six optometry students (14 males, 32 females) aged from 17 to 30 years (mean SD, 22.74 2.97) comprised the study group. Right and left eyes were measured, however only measurements from the left eye were considered for statistical analysis while the right eye acts as demonstration The ICare® was handled by an optometrist and the Goldmann tonometer by an ophthalmologist.Two measurement series, of six measurements were obtained for Icare, and the average value was recorded. For the Goldmann tonometer, two readings were obtained, and the average was recorded.

**Results** In this study, statistically significant differences were found when comparing the ICare® rebound tonometer with applanation tonometry (AT) ($p < 0.05$). The mean difference between the two tonometers was 1.34 mmHg ± 2.03 (mean ± SD) and the 95% limits of agreement were 3.98 mmHg. A frequency distribution of the differences demonstrated that in more than 80% of cases the IOP readings differed by less than 3 mmHg between the ICare® and the AT .

**Conclusion** In the present population the ICare® overestimates the IOP value by 1.34 mmHg on average when compared to Goldmann tonometer. Nevertheless, the ICare® tonometer may be helpful as a screening tool when Goldmann applanation tonometry is not applicable or not recommended, since it is able to estimate IOP within a range of ± 3.00 mmHg in more than 80% of the population.

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## The impact of ocular perfusion pressure in the retrobulbar blood flow

*MARTÍNEZ-GARCÍA A, SÁNCHEZ-SALORIO M*
*Instituto Galego de Oftalmoloxía, Santiago de Compostela*

**Purpose** To assess the impact of ocular perfusion pressure (PP) in the retrobulbar blood flow

**Methods** 16 ocular hypertensive (OHT) patients, 16 patients with open-angle glaucoma (POAG) and 14 normal control age-matched subjects were included in this study. Intraocular pressure, blood pressure and color Doppler measurements parameters in the ophthalmic (OA), central retinal (CRA) and lateral short posterior ciliary (SPCA) arteries were obtained at 9:00 and at 15:00 hours in all subjects. Correlation between Doppler parameters and PP were assessed.

**Results** The PP was lower in the OHT and POAG patients at 9:00 ($p=0.04$, $p=0.0004$) and at 15:00 ($p=0.002$, $p<0.0001$) respectively, compared with healthy subjects. Patients with glaucoma showed a higher Resistance Index in the OA, CRA and SPCA at 15:00 ($p= 0.006$, $p=0.004$ and $p<0.0001$, respectively) compared with 9:00 hour. Mean PP correlated positively with Peak systolic velocity in the OA and SPCA ($R= 0.40$, $p=0.022$; $R=0.52$, $p=0.003$, respectively) and with the End Diastolic Velocity in the OA and SPCA ($R=0.52$, $p=0.002$; $R= 0.44$, $p= 0.012$, respectively) in the POAG patients.

**Conclusion** This study suggests a defect in ocular blood flow regulation in the patients with glaucoma.

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## Pigmentary Glaucoma – Ultrasound Biomecroscopy Imaging (UBM) before and after laser iridotomy

*DA COSTA SA, COELHO A, MAIA SECO J, PRIOR FILIPE H, QUINTAO T, CASTANHEIRA-DINIS A*
*Lisbon institute of Ophthalmology Dr. Gama Pinto – Lisbon Faculty of Medicine – C.E.C.V., Lisbon*

**Purpose** The aim of our study is to show the morphological alterations of pigmentary glaucoma, using UBM images before and after YAG iridotomy.

**Methods** We imaged a group of patients with pigmentary glaucoma, before and after YAG iridotomy.

**Results** Before YAG laser, these patients exhibited contact of the posterior iris surface with the zonular fibers, a large anterior chamber and a reduction in the posterior chamber depth. After YAG iridotomy there was a marked reduction in the posterior bowing of the iris.

**Conclusion** The UBM images, after YAG iridotomy, showed a reduction in the reverse pupillary block configuration, with less contact of the zonular fibers with the posterior iris surface and a subsequent lowering in the iris pigment release, which helps in the controle of the intraocular pressure.

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## Structural changes of the anterior chamber angle in primary congenital glaucoma with respect to normal development

*SALAZAR JJ, RAMIREZ AI, DE HOZ R, ROJAS B, RAMIREZ JM, FERNANDEZ Y, TRIVIÑO A*
*Instituto de Investigaciones Oftalmologicas Ramon Castroviejo. UCM, Madrid*

**Purpose** To study the human anterior chamber angle at different stages of development, with special reference to Schlemm's canal (SC). To compare findings in normal angles with those from primary congenital glaucoma in order to clarify the pathogenic mechanisms of the disease and to explain the success of surgical treatment in some of these patients.

**Methods** Eyes from foetuses, adult normal eyes from cadavers and fragments of surgical trabeculectomies from patients with primary congenital glaucoma. Eyes were processed for examination by light microscopy, scanning electron microscopy and mesoscopy with diaphanization.

**Results** Changes in the trabecular tissue lead to formation of the angular recess and opening up of the anterior chamber angle. At 24 weeks of gestation, the SC and the post-trabecular paths emerging from its outer wall can be visualized. These run to the eye surface, forming the superficial and deep scleral plexi. In congenital glaucoma: 1) changes in the trabeculae could be responsible for the high iris insertion; 2) no observable pre-trabecular membrane but enlarged trabeculae with diminished inter-trabecular spaces are present; 3) the SC is apparently normal, with vacuoles in the wall indicating normal functioning; 4) sectioning of the abnormal trabecular tissue during goniotomy allows repositioning of the angle structures and hence the appearance of the angular recess.

**Conclusion** Anomalies of the trabecular structures in primary congenital glaucoma do not always parallel an abnormal development of the SC and the colectors channels.(Support: ISCIII-FIS CO3/13)

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## Glaucoma awareness and detection in African-Caribbeans:'Talking the talk'

*CROSS V (1), SHAH P (1), BATIVALA R (2), SII F (1)*
*(1) Ophthalmology, Birmingham*
*(2) Optometry, Birmingham*

**Purpose** Investigations into glaucoma awareness have drawn on informed, clinic-based populations. This study aimed to explore awareness in potentially less informed community outside the hospital eye service. At the same time it investigated participants' opinions and utilisation of primary eye-care services.

**Methods** Qualitative, face-to-face interviews and focus group discussions took place with twenty-four female and twenty-four male African Caribbean participants in an inner city community. Interview data were transcribed and coded using manual and computer-aided methods. Inferences and interpretations were corroborated by discussion with expert advisors and community members not directly involved in the study.

**Results** Positive attitudes to health promotion existed, but 'eye health' did not appear to be integral to individuals' family eye health narratives. The study illuminated the way eye-health behaviour is shaped by individuals' experiences and viewpoints, and indicated how ophthalmic practitioners dealing with clients and patients on a one to one basis, might be more reflective and sensitive to service users' needs and expectations in this community.

**Conclusion** The study broadened the scope for dialogue and negotiation related to African-Caribbean glaucoma. Involvement in the study motivated participants and engendered a climate of trust that should facilitate recruitment to further research on glaucoma pathogenesis and clinical outcomes in the African-Caribbean eye. However, if avoidable glaucoma blindness in young African-Caribbean people is to be prevented, 'talking the talk' of effective glaucoma detection and management must be matched by 'walking the walk' of developing flexible and responsive local eye-care services.

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## Is Primary Open-Angle Glaucoma influenced by the ambient temperature?

*WEALE RA (1, 2)*
*(1) Institute of Gerontology, King's College London, London*
*(2) University College London Hospital Eye Department, London*

**Purpose** To present evidence in favour of the view that ambient temperature is a risk factor for POAG.

**Methods** An analysis of age-related prevalence data on POAG relating to widely different geographical locationssuggests that the age of onset of the condition varies inversely with the patients' environmental temperature. Moreover, the marked difference between Afro-American patients on the one hand, and Caucasian Americans on the other, is amenable to a biological explanation. It is also found that the overall prevalence shows a statistically significant variation with temperature even if negroid patients are excluded. A tentative explanation for the hypothesized role of temperature will be presented as it offers some quantitative support for the above notion.

**Results** It is possible to conclude from published data that the prevalence of POAG varies statistically significantly with temperature.

**Conclusion** The average annual ambient temperature in which a sample population find themselves may be a risk factor for POAG.

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## A Comparison of Travatan® and Cosopt® Therapy in Patients with Open Angle Glaucoma or Ocular Hypertension

*SUZUKI JUNIOR ER, MEIRELLES LF, FIGUEIREDO CRL, AGOSTINI NETTO J, BATISTA WD*
*Glaucoma, Belo Horizonte*

**Purpose** The purpose of this study was to compare Travatan® (TRAV; travoprost 0.004%) and Cosopt® (COS; dorzolamide 2.0%/timolol maleate 0.5%) ophthalmic solutions for reducing intraocular pressure (IOP) in patients with open angle glaucoma or ocular hypertension patients.

**Methods** This was an open label, parallel, randomized study. Baseline IOP measurements ranged from 21 to 36 mmHg at 8:00 AM. The TRAV group (n = 29) dosed once daily at 9:00 PM while the COS group (n = 27) dosed twice daily at 9:00 AM and 9:00 PM. IOP was measured at screening, and following 3 weeks and 6 weeks of treatment at 8:00 AM, 12:00 PM, 4:00 PM and 8:00 PM. A quality of life analysis was also conducted.

**Results** Mean daily average IOP reductions from baseline were 7.5 (32.7%) and 7.1 (30.7%) mmHg for TRAV and 4.8 (23.1%) and 4.5 (21.7%) mmHg for COS at 3 weeks and 6 weeks, respectively averaged across all time points. The greater IOP reduction for TRAV was statistically significant at both the 3 and 6 week visits when averaged across all time points ($P < 0.01$). The two products were safe over the course of six week study. Some factors such as bad taste and dry mouth were worse in the COS group. Most patients (58.5%) preferred once daily dosing, while nearly 70% of patients reported that using eyedrops only once a day helped them to remember to take their medication.

**Conclusion** Both TRAV and COS were safe and effective in lowering IOP. Reductions in IOP were greater for TRAV than for COS. The patient preference evaluations favored the medication with once a day dosing.

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## IOP-REDUCING EFFECT OF TRAVATAN® VS XALCOM® IN PATIENTS PREVIOUSLY TREATED WITH TOPICAL B-BLOCKERS

*FRANKS W (1), RENARD JP (2)*
*(1) Glaucoma Unit. Moorfields Eye Hospital., London*
*(2) Service d'ophtalmologie.Hôpital du Val de Grâce., Paris*

**Purpose** To evaluate the effect on intraocular pressure (IOP) of TRAVATAN® (TRAV; travoprost 0.004% ophthalmic solution) versus XALCOM® (XC; latanoprost 0.005%/timolol 0.5% fixed combination ophthalmic solution) in patients with open angle glaucoma (OAG) or ocular hypertension (OH) where IOP was previously treated with topical β-blocker therapy.

**Methods** 110 patients (on stable treatment with β-blockers and mean IOP between 22 and 36 mmHg at 9 AM in at least one eye) were randomised to TRAV or XC, received medication and were evaluable for safety. The dosage regimen was one drop in each eye once-daily for 6 weeks. The efficacy was evaluated by comparing IOP at 9 AM and 5 PM at baseline and following 2 and 6 weeks of treatment.

**Results** At baseline, there were no significant differences between the treatment groups in terms of age, gender, ethnicity, iris color, or diagnosis. Between-treatment differences in IOP for the four on-therapy visits and times ranged from -0.7 to +0.6 mmHg (P ⊠ 0.3377). Mean IOP reductions from baseline were up to 7.3 mmHg (29%) for TRAV and up to 6.5 mmHg (26%) for XC. No serious, related, and unexpected events were reported for either of the treatment groups.

**Conclusion** TRAV and XC produce similar IOP-lowering efficacy in patients with OAG or OH who are previously treated with topical β-blocker therapy.

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## Effect of latanoprost on the anterior chamber depth and its dynamics in patients with glaucoma or ocular hypertension

*GUTIERREZ C (1), TELIS MA (2, 3, 4)*
*(1) Hospital Universitario Príncipe de Asturias, Alcalá de Henares, Glaucoma, Madrid*
*(2) Hospital Universitario Príncipe de Asturias, Alcalá de Henares, Madrid*
*(3) Universidad de Alcalá de Henares, Madrid*
*(4) VISSUM, Madrid*

**Purpose** To evaluate the effect of latanoprost on the depth of the anterior chamber and its dynamics in patients with high intraocular pressure.

**Methods** A prospective, nonrandomized comparative trial that included 10 patients (20 eyes) with glaucoma or ocular hypertension that were treated during 1 month with Latanoprost was carried out. The depth of the anterior chamber with ultrasonography was measured before and after treatment. In addition, the anterior chamber's deep was measured before and after half an hour the instillation of pilocarpine 2% before and after one month of treatment. No previous treatment has been prescribed to the patients.

**Results** The mean deep of the anterior chamber before the treatment and before the instillation of pilocarpine was 3.34mm (SD:0.51) and following the instillation was 3.19mm(SD:0.5). After 1 month of treatment, it was 3.22mm (SD:0.50) before pilocarpine and 3.1mm (SD:0.57) after pilocarpine. The differences were statistically significant ($p<0.05$) for all comparisons.

**Conclusion** These findings suggest that prostaglandins decreased the dept of the anterior chamber in patients with glaucoma or ocular hypertension. These agents might relax the zonular fibres or, more probably, increase the ciliary muscle contraction.

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## Argon laser trabeculoplasty versus selective laser trabeculoplasty: short term outcome

*VAN DE VEIRE S, ZEYEN T, STALMANS I*
*Department of Ophthalmology, University Hospitals, Leuven*

**Purpose** To compare argon laser trabeculoplasty (ALT) with conventional selective laser trabeculoplasty (SLT) in terms of their efficiency in lowering the intra-ocular pressure.

**Methods** In this retrospective study, 56 eyes from 44 patients with primary open angle glaucoma, ocular hypertension, pseudo-exfoliative or pigment dispersion glaucoma (PDG) were included. Patients underwent either ALT (n=38) or SLT (n=18). The intra-ocular pressure (IOP) was measured immediately prior to and 3 to 5 weeks after the therapy.

**Results** At 3 to 5 weeks, there was a non-significant trend towards more IOP-reduction after ALT: 22.4% versus 15.5% after SLT (p= 0.141). Of note, of the four patients with PDG that were included, 2 underwent ALT and 2 SLT. Remarkably, both patients who underwent SLT showed a paradoxical rise in IOP after the procedure (+15.5%). When these patients were excluded from the analysis, a similar hypotensive efficacy was found between ALT (19%) and SLT (17.9%)(p= 0.836). A small study with lower energy levels (<0.9mJ) confirmed this observation again in 6 patients. It occurred in the absence of steroid treatment and persisted until 12 weeks after treatment.

**Conclusion** The short term efficacy of ALT and SLT was similar. In this study, the patients with PDG who underwent SLT showed a paradoxical rise in IOP. This finding may indicate that even lower energies are required when performing SLT in patients with heavily pigmented trabeculae and merits further investigation in a larger scale prospective study on the effect of ALT versus SLT in this type of glaucoma.

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## Lasering the trabecular meshwork and limbal veins in adult rats results in ocular hypertension and retrograde degeneration of retinal ganglion cells

*VIDAL-SANZ M (1), MAYOR S (1), RAMIREZ AI (2), TRIVIÑO A (2), SALAZAR JJ (2), RAMIREZ JM (2), SALINAS M (1), VILLEGAS-PEREZ MP (1)*
*(1) Oftalmología, Murcia*
*(2) Instituto Castroviejo, Madrid*

**Purpose** To study the effects of chronic ocular hypertension, induced by laser photocoagulation of episcleral and limbal veins, on the survival of adult rat retinal ganglion cells (RGC).

**Methods** In adult Sprague-Dawley rats, the intraocular pressure of the left eye was chronically elevated by laser photocoagulation (532nm wavelength, 500mW power, 0.25 second duration, 100 µm diameter spot size) of limbar and episcleral veins. Intraocular pressure in both eyes was measured with a Tono-Pen prior to and 1 and 2 weeks after lasering. Retinal ganglion cell survival was investigated by examining RGCs labeled with fluorogold applied to both superior colliculi 1 week before sacrifice and with dextran tetramethylrhodamine (DTMR) 3000 molecular weight (MW) applied intraorbitally to the ocular stump of the transected left optic nerve 5 days after FG application and 2 days prior to sacrifice. The optic nerve and the nerve fiber layer was examined with neurofilament antibodies (RT97).

**Results** Laser treatment resulted 1 and 2 weeks an increased IOP of approximately 180 and 136%, respectively, over baseline. Three weeks after lasering, there were wedge sectors of the retina lacking RGCs retrogradely labelled with FG. The neurofilament staining in these sectors revealed the presence of RGC axons that showed typical features of axonal degeneration. There was certain variability among different animals in the size and location of these sectors.

**Conclusion** Elevation of the intraocular pressure results 3 weeks later in RGC loss and altered retrograde axonal in pie shaped sectors of the retina.

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## Our experience of using semiconductor laser for the treatment of neovascular glaucoma after diabetic retinopathy in Armenia

*BUNIATYAN IRINA Y*
*Ophthalmologic centre n.a. S.V.Malayan, Glaucoma department, Yerevan*

**Purpose** Transscleral cyclophotocoagulation is a procedure for glaucoma that is performed on an outpatients basis. Several types of glaucoma are associated with diseases of the retina. Approximately one-third of the patients with rubeosis iridis have diabetic retinopathy. We used the semiconductor diode laser to perform transscleral cyclophotocoagulation in 15 eyes of 15 patients with neovascular glaucoma with pain syndrome after diabetic retinopathy.

**Methods** The power applied was 1500-2000 mW; duration 1500-2000 msec, number of coagulants 21 (average) by circomference (depending on IOP and pain syndrome intensity by VAS). The operation was performed with IRIS Oculight Diode Photocoagulator with G-probe, Medical Instruments, Inc.The cyclophotocoagulation procedure was performed with local anesthesia. After the procedure the patients started anti-inflammatory eye drop medications and oral anti-inflammatory medications.

**Results** The initial average IOP level by Goldmann was 43,87 mm Hg and the mean intraocular pressure 3 months after a single treatment session was 24,8 mm Hg and after a year-23.4 mm Hg. The pain syndrome was gone in all patients. The next day after TSCPC IOP increased in 3 cases (20%) and decreased to normal level by Goldmann (below or equal to 26 mm Hg) with 2 patients (13.33%), after 90 days normal level of IOP was in 9 cases (60%) and after 1 year and more in 11 cases (73.33%).

**Conclusion** These results suggested that semiconductor diode laser transscleral cyclophotocoagulation may be useful in the treatment of neovascular glaucoma after dibetic retinopathy to reduce the IOP and pain syndrome.

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## Trans-scleral Diode Laser Cyclophotocoagulation treatment for refractory glaucoma

*PHERWANI AA (1), GREGORY ME (2), SIM K (1)*
*(1) Ophthalmology, Birmingham and Midland Eye Centre, Birmingham*
*(2) Ophthalmology, University Hospital of of North Staffordshire, Stoke on Trent*

**Purpose** To analyse the results obtained following trans-scleral diode laser cyclophotocoagulation treatment of refractory glaucoma

**Methods** A retrospective analysis of the case notes of 54 consecutive patients who underwent cyclodiode laser between January 2002 and March 2004 was performed.

**Results** 60 eyes were treated. Mean pre-treatment intra-ocular pressure (IOP) of 37.71mmHg (SE ±1.6mmHg) was reduced to 25.49mmHg (SE ±1.77mmHg) at the final index visit. Following treatment, 52% achieved an IOP of less than 22mmHg, 52% had over 30% drop in their IOP, and pain was relieved in 11 of 13 patients (84.6%). The mean topical antiglaucoma medication score was reduced from 2.17 to 1.88, with oral acetazolamide stopped in 11 of 15 patients (73.3%). 17 eyes (28.3%) were re-treated, with the mean number of treatments being 1.36. Hypotony occurred in one eye (1.6%). There was an overall success rate of 65.5%, with success being defined as IOP less than 22mmHg, over 30% reduction in IOP with or without medication, discontinuation of oral acetazolamide, or pain relief.

**Conclusion** Cyclodiode laser was highly effective in relieving pain and stopping oral acetazolamide, but less satisfactory in reducing IOP, possibly due to inadequate re-treatment.

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## A Little Bit of ReGAE (Research into Glaucoma and Ethnicity) – The Story So Far with Trabeculectomy and Mitomycin C

*SHAH P, SII F, LOCKWOOD A*
*Ophthalmology, Birmingham*

**Purpose** To determine the efficacy and safety of trabeculectomy with mitomycin C to control intraocular pressure (IOP).

**Methods** Phase 3 of the Birmingham ReGAE Project is an open, prospective and consecutive series of all patients who had undergone trabeculectomy with mitomycin C. The surgery was performed by a single surgical team at the Birmingham and Midland Eye Centre.

**Results** One hundred and sixty-nine eyes of 139 patients (53.2% males) with a mean age of 55.9 years were included. These patients were Caucasians (73.4%), African-Caribbeans (20.7%) and Asians (5.9%). The mean of preoperative highest and mean IOPs were 35.6 and 22.9 mm Hg, respectively, compared with the mean of postoperative IOP of 13.0 mm Hg at the last follow-up. When the patients were stratified for the postoperative response, there were 95.3%, 88.2%, 72.8% eyes with IOPs of ≤ 21, ≤ 17 and ≤ 14 mm Hg, respectively. 68.0% of eyes achieved IOP reduction > 30% from the preoperative mean IOP. The overall mean % of IOP reduction from mean preoperative IOP was 39.5%. The number of topical medications was averaging 3.1 (35.9% on acetazolamide) preoperatively, reducing to an average of 0.2 (3.6% on acetazolamide) postoperatively. There were 17.8% of eyes that required blebneedling revisions postoperatively. Complications encountered in this series were clinically significant early hypotony (6.5%), hyphaema (5.9%), late hypotony (0.6%), cataracts requiring extractions (1.8%), iris incarceration (0.6%) and endophthalmitis (0.6%).

**Conclusion** In a prospective consecutive series of patients undergoing trabeculectomy with mitomycin C, the procedure has excellent safety and efficacy profiles.

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## Impiant Ex-PRESS miniature glaucoma under a scleral flap

*CARNOVALE SCALZO G, SCORCIA V, BRUZZICHESSI D, CATALANO C, SCORCIA G*
*Eye Clinic, University of Catanzaro "Magna Graecia", Catanzaro*

**Purpose** The aim of this study was to test the safety and efficacy of the Ex-PRESS miniature glaucoma device when it is implanted under a scleral flap in patients affected to neovascular glaucoma

**Methods** The Ex-PRESS implant was inserted into the anterior chamber under the scleral flap at the limbus in 40 eyes of 40 patients with neovascular glaucoma (occlusion of the vein central, diabetic retinopathy) in maximal. hypotensive therapy

**Results** The intraocular pressure was significantly reduced from 40 +/- 3 mm Hg preoperatively to 13 +/- 5.0 mm Hg at 12 months postoperatory, only two patients needed anti-glaucoma medications to keep the IOP below 30 mm Hg.

**Conclusion** Secondary glaucoma to vascular causes (occlusion of the vein central, diabetic retinopathy) represented a contraindication to the system of Ex-PRESS systems under the congiuntiva our study has demonstrated that the Ex-PRESS system under to scleral flap can be one solution in the cases of refractory glaucoma

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## Pneumatic Trabeculoplasty (PNT): our experience

*PESCE G, FRATTO M, SCHIAVELLO M, SCORCIA V, BRUZZICHESSI D, CARNOVALE SCALZO G*
*Eye Clinic, University of Catanzaro "Magna Graecia", Catanzaro*

**Purpose** The aim of this study was to determine the efficacy and the safety of Pneumatic Trabeculoplasty (PNT) alone to lower IOP in with primary open angle glaucoma (POAG) or ocular hypertension (OH).

**Methods** 20 POAG or OH patients (10 males, 10 females) with range IOP 19-24 mm-Hg on monotherapy or without any treatment. The follow up to 1-7-30-60-90- 120 days post PNT treatment it includes the evaluation visual acuity,biomicroscopy, applanation tonometry,visual field, gonioscopy and ophthalmoscopy

**Results** The results was showed a mean IOP reduction of 9,30%. post PNT treatment

**Conclusion** This study of PNT showed a potentially good IOP lowering effect on glaucoma and hypertensive patients.

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## Who Will Need Bleb Needling Revision with 5FU After Trabeculectomy with Mitomycin C?

*SII F, SHAH P, LOCKWOOD A*
*Ophthalmology, Birmingham*

**Purpose** To study the characteristics of patients/eyes that required bleb needling revisions with subconjunctival injections of 5-fluorouracil (BNR-5FU) after trabeculectomy with mitomycin C

**Methods** The Birmingham ReGAE (Research into Glaucoma and Ethnicity) Project is an open, prospective, multi-phase, multi-disciplinary and ethnically-sensitive consecutive study in which Phase 3 assesses the surgical outcomes of trabeculectomy with mitomycin C. The decision made to perform BNR-5FU was based on either suboptimal intraocular pressure (IOP) control, and/or suboptimal bleb morphology. The characteristics of these patients and eyes were identified.

**Results** BNR-5FU was performed on 30 eyes (64.5% vs 48.9% males) of 169 eyes that had undergone trabeculectomy with mitomycin C. Of the 30 eyes, there were 26.7% African-Caribbeans, 6.7% Asians, and 66.7% Caucasians (compared with 19.4%, 5.8%, 74.8%, respectively in the group of 139 eyes that had not had BNR-5FU). Eight (26.7%) patients (vs 23.7%) were younger than 45 years of age. Seventeen (53.3%) eyes (vs 38.1%) have glaucoma other than primary open angle or normal tension glaucoma. Fourteen (46.7%) eyes (vs 25.9%) had previous intraocular surgery. The mean of highest preoperative IOP was 40.9 (vs 34.4) mm Hg, respectively. Twenty six (86.7%) eyes (vs 60.0%) had highest preoperative IOP of ≥ 30 mm Hg and 50.0% (vs 32.4%) were on oral acetazolamide

**Conclusion** The characteristics of patient/eyes that predict the likelihood of postoperative BNR-5FU were identified. These were male gender, previous intraocular surgery, glaucoma types other than normal tension or primary open angle glaucoma, highest preoperative IOP of ≥ 30 mm Hg, and the requirement of preoperative oral acetazolamide to control IOP

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## Safety and Efficacy of Trabeculectomy and Mitomycin C in Uveitic Glaucoma

*LOCKWOOD A (1), SII F (1), CHIANG M (2), SHAH P (1)*
*(1) Ophthalmology, Birmingham*
*(2) Ophthalmology, Brisbane*

**Purpose** To determine whether trabeculectomy with mitomycin C (MMC) can be used with good effect and safety to control intraocular pressure (IOP) in patients with uveitic glaucoma.

**Methods** An open consecutive prospective study was performed on 22 eyes of 19 patients with uveitic glaucoma who underwent trabeculectomy with MMC for management of uncontrolled IOP despite maximally tolerated medical treatment. The following uveitic diagnoses were present: Fuchs uveitis (8 eyes), idiopathic inflammation (11 eyes), sarcoidosis (2 eyes) and ankylosing spondylitis (1 eye). All patients had strict uveitis control at the time of surgery.

**Results** The mean length of follow-up from the time of surgery to the last visit was 13.3 months. At the last follow up examination 21 (95.5 %) of the trabeculectomies met the criteria for qualified success and 20 (91 %) met the criteria for complete success. Twelve patients reached 12 months follow up and of these 11 (91.9 %) achieved an IOP of 17 mm Hg or less, 10 (83.3 %) achieved an IOP of 14 mm Hg or less and 10 (83.3 %) achieved a 30 % reduction in IOP. Best-corrected visual acuity improved or remained within two lines of preoperative visual acuity in 20 eyes (91 %). One (4.5 %) patient developed a bleb leak with early hypotony and transient worsening of intraocular inflammation. Two patients (9 %) developed a transient hyphaema. One (4.5%) patient developed a transient vitreous haemorrhage.

**Conclusion** Trabeculectomy with intraoperative application of MMC can be used with good efficacy and with few complications in this group of complex refractory patients. Meticulous control of preoperative inflammation and careful surgical technique with active postoperative bleb manipulation all contribute to the excellent success rate of surgery.

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## Anterior Segment Optical Coherence Tomography of the Ex-PRESS miniature glaucoma implant

*BIALLOSTERSKI C (1), DE BRUIN DM (2), AALDERS M (2), FABER DJ (2), DE JONG LAMS (1), VERBRAAK FD (1, 2)*
*(1) Ophthalmology, AMC, Amsterdam*
*(2) Laser Centre, AMC, Amsterdam*

**Purpose** To describe the localisation of the Ex-PRESS miniature glaucoma implant with an experimental setup for optical coherence tomography (OCT) of the anterior segment of the eye.

**Methods** An OCT scanner, central wavelength 1,280 nm, bandwidth 60 nm, resolution of 12 µm, was built onto a slitlamp to scan the anterior segment of the eye. Five ex-vivo porcine eyes received an Ex-PRESS miniature glaucoma implant and were used as a model to visualise the position of the implant in the anterior segment. Several patients with the device were also scanned according to the same protocol as well as several normal eyes.

**Results** In the ex-vivo porcine eyes, the OCT images showed the anatomy of the anterior segment in great detail. The anterior segment OCT was able to visualise the whole outline and position of the implant. The abrupt change in reflectivity going from tissue to the implant resulted in a clear border along the circumference of the whole device. The first results in patients with the implant proved to be more difficult to obtain because of motion artefacts.

**Conclusion** In this paper, we have shown that we were able to outline the Ex-PRESS miniature glaucoma implant in the anterior segment of the ex-vivo porcine eye by using an experimental OCT setup built onto a slitlamp. Due to motion artefacts it is more difficult to visualise the implant in the in vivo human eye. The anterior segment OCT is expected to aid in the definition of the parameters determining the success or failure of such a device.

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## Deep sclerectomy suplemented with 5 Fluorouracil

*GUTIERREZ C (1), TEUS MA (2, 3, 4)*
*(1) Hospital Universitario Príncipe de Asturias, Alcalá de Henares, Glaucoma, Madrid*
*(2) Hospital Universitario Príncipe de Asturias, Alcalá de Henares, Madrid*
*(3) Universidad de Alcalá de Henares, Madrid*
*(4) VISSUM, Madrid*

**Purpose** To study the outcome of deep sclerectomy supplemented with 5-Fluorouracil in patients with open angle glaucoma.

**Methods** Prospective consecutive series of 28 patients with medically uncontrolled open-angle glaucoma undergoing deep sclerectomy supplemented with 5-Fluorouracil (5 minutes, 50 mg/ml) under the conjuntival flap. All patients underwent clinical assessment before and after surgery at days 1 and 7 and at months 1, 3, 6, 9 and 12. Surgical success was considered if the patient's intraocular pressure (IOP)<21 mmHg with or without the use of medication.

**Results** The mean follow-up time was 6.28 months (range:3-12 months). Mean IOP decreased significantly from a preoperative value of de 21.08 mmHg (SD:3.59) mmHg to a postoperative value of 16 (SD: 3.53) at 12 months (P<0.001). At the end-point of follow-up success was found in the 85% of the patients without treatment and in the 92.85% with treatment. Complications were the followings: hiperfiltration (1 patient), Seidel (3 patients) and encapsulated bleb (1 patient). YAG laser was performed in the 25% of the patients. There were no other late complications.

**Conclusion** Deep sclerectomy supplemented with 5 Fluouracil has a high rate of IOP reduction and a low rate of complications.

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## Preclinical assessment of a radioactive implant for glaucoma filtering surgery – preliminary results

*PICHLER A (1), HELD A (1), SCHUBERT M (2), ASSMANN W (2), LANZL IM (1)*
*(1) Department of Opthalmology, Technische Universität München, Munich*
*(2) Physics Department, Ludwig-Maximilians-Universität München, Munich-Garching*

**Purpose** Glaucoma filtering surgery is most commonly performed in the form of trabeculectomy (TE). Despite due surgical care TE has a failure rate due to tenon fibroblast proliferation of up to 30 percent within 3 months and 50 percent within a year. The modulation of wound healing in glaucoma filtering surgery is a major research task. A biodegradable polylactide (PLGA) radioactive implant inducing local modulation of wound healing could represent a new approach to inhibit the undesired scarring in glaucoma filtering surgery.

**Methods** A conventional TE was performed in New Zealand white rabbits. PLGA (2x2x0,3mm) was implanted under a 3x3 mm scleral flap in seven animals. Seven further animals received the same implant with a 32P modified surface (15 Gray). During the three postoperative weeks the intraocular pressure (IOP) was controlled by TonopenXL. Histology was performed 3 weeks after surgery.

**Results** Group 1: IOP difference between operated and unoperated eye one week post op 2,0 +/- 1,8 mmHg, two weeks post op 0,7 +/- 1,8 mmHg, three weeks post op -0,5 +/- 2,6 mmHg. Group 2 (radioactive implant): IOP one week post op 1,0 +/- 2,5 mmHg, two weeks post op 1,4 +/- 1,3 mmHg, three weeks post op 0,9 +/- 1,5 mmHg. Histology: In all eyes a formed space could be detected underneath the scleral flap. PLGA was almost completely resorbed. Only few lymphocytes could be detected.

**Conclusion** Our results show no statistically significant differences in postoperative IOP. Because of the very thin cornea of the rabbit eye the IOP as measured by TonopenXL has a high variation. Immunohistochemistry is performed to differenciate the cellular response after the two different implants.

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### Central corneal pachymetry in children with congenital glaucoma after trabeculotomy

*OBERACHER-VELTEN I, LORENZ B*
*Dept. of Paediatric Ophthalmology, University Eye Hospital, Regensburg*

**Purpose** It was the aim of the present study to investigate the value of corneal pachymetry in children with congenital glaucoma after trabeculotomy.

**Methods** Twelve eyes of nine children with congenital glaucoma had follow-up examinations with corneal ultrasound pachymetry, applanation tonometry, and slit-lamp biomicroscopy before and for at least three months after trabeculotomy. Corneal pachymetry, slit-lamp biomicroscopy, and whenever possible applanation tonometry were performed without general anaesthesia.

**Results** Seven of twelve eyes had biomicroscopically clear corneas without visible corneal edema before trabeculotomy. Mean central corneal thickness was 680 ± 57 µm before trabeculotomy and decreased to 597 ±45 µm after trabeculotomy. Mean intraocular pressure (IOP) was 22 ± 5 mmHg before and decreased to 12 ± 4 mmHg after trabeculotomy. The decrease of central corneal thickness was significantly correlated with the decrease in IOP. Even those eyes with biomicroscopically clear corneas before trabeculotomy showed a decrease in central corneal thickness after trabeculotomy.

**Conclusion** Central corneal pachymetry seems to be a useful additional information in children with congenital glaucoma after trabeculotomy. As corneal ultrasound pachymetry easily could be performed without general anaesthesia in all children and often can be done more easily than applanation tonometry, it can be taken as additional follow-up examination to control the IOP regulation in children with congenital glaucoma.

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### Retrobulbar hemodynamics in asymmetric glaucoma

*REMKY A, PLANGE N, KALIP M, AREND O*
*Universitäts-Augenklinik, Technical University Aachen, Aachen*

**Purpose** Previous studies investigated blood flow velocities of retrobulbar vessels in patients with glaucoma. Most of the studies confirmed reduced end-diastolic velocities associated with elevated resistive indices in the central retinal artery and the posterior ciliary arteries. This study was performed to correlate the degree of glaucomatous damage with retrobulbar hemodynamics in patients with normal tension glaucoma (NTG) and primary open-angle glaucoma (POAG) by comparing in the same patient the eye with advanced visual field loss with that with no ore mild field loss.

**Methods** 25 POAG patients and 15 NTG patients exhibiting asymmetric visual field loss (difference of mean deviation between the eyes >6dB) were included in this prospective 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 color Doppler imaging in both eyes.

**Results** Mean deviation of eyes with more severe glaucomatous visual field loss was –17.1dB versus –5.6dB ($p<0.0001$). Intraocular pressure and perfusion pressure were not significantly different between eyes. The PSV of the OA (30.5 ±8cm/s versus 32.9 ±9cm/s, $p=0.014$) and the EDV of the CRA (2.2 ±0.4cm/s versus 2.5 ±0.5cm/s, $p<0.005$) were significantly decreased in eyes with more severe glaucomatous field loss. The diagnosis (either POAG or NTG) had no influence on hemodynamic data.

**Conclusion** Patients with POAG or NTG and asymmetric glaucomatous visual field loss exhibit asymmetric blood flow velocities of the OA and CRA. The posterior ciliary arteries did not show any significant differences between the more and less affected eye.

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### A new scoring system for giant cell arteritis (GCA) - Can the result of a temporal artery biopsy (TAB) be predicted?

*NESTEL AR, WHITTAKER KW, GIBSON RA, ENOCH BE*
*North Devon District Hospital, Department of Ophthalmology, Barnstaple, Devon*

**Purpose** To establish a new test for the diagnosis of GCA based on a clinical scoring system and determine its predictive accuracy relative to the outcome of TAB

**Methods** A score chart was devised, with each symptom and sign being allotted a numerical value, ranging from -2 to +3, according to its significance. The numerical values were derived from an extensive literature review and fine-tuned based upon an a initial review of 20 consecutive cases of suspected GCA in whom a biopsy had been performed. At a score of > or = 6 the test was considered positive, at a score of <6 the test was considered negative (- the hypothesis being that a greater proportion of patients with GCA would have scores of > or = 6 compared with patients without GCA). A blind study was then conducted on further 35 consecutive cases in order to determine the test's predictive accuracy.

**Results** The test had a sensitivity of 100%, a specificity of 90 %, a negative predictive value of 100% and a positive predictive value was 50% ($p<0.01$).

**Conclusion** By virtue of its high sensitivity, the scoring system appears to be a very 'safe' test, successfully identifying all cases of biopsy-proven GCA. This is critical given the potentially devastating nature of the disease. Its excellent negative predictive value was such that, had it been applied prospectively in this series, 82% of TAB could have been avoided. Taken together these results suggest that the scoring system could provide an effective predictive aid. A larger prospective study may be helpful in further establishing the clinical role of such a scoring system.

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### Intraocular Pressure Lowering effect of dorzolamide/timolol fixed combination in non-responder glaucoma patients to prostaglandin analogues/prostamides

*MARTÍNEZ-GARCÍA A, SÁNCHEZ-SALORIO M*
*Instituto Galego de Oftalmoloxía, Santiago de Compostela*

**Purpose** Purpose: To evaluate the effect of the dorzolamide/timolol fixed combination (DTFC) in non-responder glaucoma patients to prostaglandin analogues/prostamides.

**Methods** Methods: 21 patients (1 eye from each) with open-angle or capsular glaucoma were included in this retrospective observational cohort study. 119 consecutive glaucoma patients treated with prostaglandin analogues/prostamides were screened, between June 2003 and December 2004, 21 patients (17.6%) were considered Non-responder. Non-responder was defined as an intraocular pressure (IOP) lowering effect less than 15% compared with baseline measurement. IOP was measured at 8 AM, 10 AM, 12 AM, 2 PM, 4 PM, 6 PM, 8 PM and 10 PM at baseline and at the end of each treatment period.

**Results** Results: At baseline the Highest IOP (mean (SD)) was 27.67 (3.26) mm Hg at 12AM and the lowest IOP was 20.67 (1.83) mm Hg at 4 PM. The IOP was higher at 4PM ($p=0.035$) after treatment with prostaglandin analogues/prostamides compared with baseline measurement. The IOP was lower ($p<0.0001$) in all IOP-time point measured after treatment with the DTFC compared with prostaglandin analogues/prostamides treatment phase.

**Conclusion** Conclusion: DTFC showed a good IOP lowering effect in Non-responder prostaglandin analogues/prostamides glaucoma patients.

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## Safety and efficacy of the Optonol DS version microtube implanted during deep sclerectomy in POAG patients: preliminary results

*POURNARAS J-A C, MERMOUD A*
*Jules Gonin Eye Hospital, Lausanne*

**Purpose** To assess the safety and efficacy of the Optonol DS version microtube implanted during deep sclerectomy in POAG patients.

**Methods** This non-randomized, prospective pilot study include 5 POAG patients (6 eyes) requiring filtering surgery. Deep sclerectomy was performed and the miniature implant was inserted at the anterior part of the deep sclerectomy into the anterior chamber. The complete eye evaluation was performed before and after surgery during 6 months. UBM were also performed.

**Results** At the sixth month, the mean postoperative intraocular pressure was significantly reduced from 21.14 ± 2.41 mmHg to 12 ± 2.7 mmHg (p=0.0019; n=6). All implants were correctly implanted, as shown by the UBM.

**Conclusion** Optonol DS version microtube inserted during deep sclerectomy is well tolerated and significantly reduces the intraocular pressure at the sixth month without any notable complication.

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## The feasibility of implantation of pseudophakic wide angle miniature telescope in eyes with ruptured lens capsular bag

*ROSNER M, SACHS D, ZIV H, BELKIN M*
*Goldschleger Eye Research Institute, Sackler School of Medicine, Tel-Aviv University, Sheba Medical Center, Tel-Hashomer*

**Purpose** The Implantable Miniature Telescope (IMT by Dr. Lipshitz, VisionCare Ophthalmic Technologies Ltd. Yehud) was developed to improve the central vision of patients with bilateral AMD. It was designed for implantation in the capsular bag. However, in many cases the capsular bag is not intact or is missing. For these cases the Pseudophakic Wide Angle modification of the telescope (PWA) was designed. The objective of this preclinical eye study was to evaluate the feasibility of PWA implantation in animal eyes without an intact capsular bag. This study preceded the preliminary clinical investigation of the PWA that is currently underway.

**Methods** PWAs were implanted in the sulcus of 20 eyes of WNZ rabbits after ECCE and rupture of the posterior capsule. In 6 eyes no additional fixation procedure was performed, trans-scleral fixation was performed in 7, and a modified scleral fixation (net) was performed in another 7 eyes. After 6 months follow-up, the rabbits were sacrificed and the eyes were removed for gross and histological evaluation.

**Results** Appropriate positioning of the device was found after 6 months in 4/6 eyes with no additional fixation, in 3/5 with scleral fixation and in 6/7 with net fixation. The mean PWA - Cornea distance was the shortest with trans-scleral fixation (3.5 mm), and the longest (4.2 mm) with net fixation (p=0.03).

**Conclusion** It is safe and feasible to implant the PWA device in the sulcus when the posterior capsular bag is not intact, and it stays centered and distant from the cornea. Either trans-scleral or net fixation may be indicated when it is estimated during surgery that the capsular remnants are insufficient to hold the PWA device secure in the sulcus.

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## Black opaque intraocular lens implantation in three patients: diagnoses leukocoria; loss of fusion; and alternating hypotropia with image delay

*DUBOIS VDJP (1), MAVRIKAKIS I (2), VICKERS S (1), LIU C (1)*
*(1) Sussex Eye Hospital, Brighton*
*(2) Ophthalmology, East Grinstead*

**Purpose** we present 3 patients who received black intraocular lenses (IOLs). we review their individual unique scenarios, with their culmination in near identical surgical procedures.

**Methods** A 71 year-old man presented with deteriorating vision in his only good eye. the other eye had suffered a traumatic retinal detachment forty years before. he had leukocoria which bothered him. he underwent immedeiately sequential right-sided cataract extraction and implant followed by phakic implantation of a black intraocular lens in the other eye.A 51 year old woman with multiple sclerosis, had had optic neuritis in her left eye which left her with an image delay. she had a subsequent episode of demyelination which left her with a skew deviation and dizziness to the extent of having to occlude her left eye(which was seeing 6/12) with a black IOL.patient 3 was a middle aged hyperopic man who suffered a head injury in his late teens which left him with an inability to fuse images together such that he constantly saw double 'like begin really drunk' he has remained symptomatic for the last 35 years. VA 6/6 in both eyes.no orthoptic exercises nor prisms could relieve his symptoms. occlusive contact lens only worked when supplemented by the palm of his hand. he is currently recovering from surgery.

**Results** This poster reviews three unique patients, who ended up with near identical surgical procedures.

**Conclusion** remember that black IOLs are a semi-permanent choice of menagement, and expose the patient to the risk of phthisis, so always explore non-surgical intervention such as corneal tattoos and occlusive contact lenses first. this is all the more true for eyes seeing 6/6 or better!

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## Correlation between IOL materials and secondary cataract. A flare meter study

*BRAVETTI GO (1), CELLINI M (1), BENDO E (1), CAMPOS EC (2)*
*(1) Ophthalmology Service-S.Orsola Malpighi, Bologna*
*(2) Ophthalmology Service, Bologna*

**Purpose** To determine the influence of posterior chamber intraocular lens implantation on the incidence of secondary cataract and the grade of anterior chamber inflammation following cataract surgery.

**Methods** Sixty six patients (36 men and 30 women), between 71 and 84 age (mean 75.8 yrs) underwent cataract surgery, phacoemulsification and implantation of an intraocular lens performed by the same surgeon. We used three type of lens materials: PMMA ( 26 patients), Acrylic-hydrophobic (22 patients) and Acrylic-hydrophlic (18 patients). After 12 months in all patients we evaluated the visual acuity, the posterior capsule opacification and the the flare in the anterior chamber with a laser flare-meter (Kowa FM500, Tokio-Japan).

**Results** At 12 months we observed secondary cataract in 64% of patients examined. The grade of secondary cataract was 0.371 ± 0.298 (Acrylic-hydrophobic), 0.486 ± 0.146 (Acrylic-hydrophilic) and 0.943 ± 0.533 (PMMA). There was a statistical significantly difference between Acrylic-hydrophobic lens vs PMMA ($p<0.05$) The laser flare-meter was: 5.900 ± 2.651 photons/mm (Acrylic-hydrophobic), 6.140 ± 1.702 photons/mm (Acrylic-hydrophilic) and 10.143 ± 3.426 photons/mm (PMMA)It was statistical significantly the difference of laser flare between Acrylic-hydrophobic and PMMA ($p<0.021$). With the Spearman correlation test we have found a correlation between the anterior chamber flare and the secondary cataract in the PMMA group ($p<0.045$)

**Conclusion** The results demonstrate that the lens type have a role in the development of the secondary cataract. The higher incidence of secondary cataract was observed with PMMA maybe correlated with lens biocompatibility.

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## Quality of vision after AcrySof ReSTOR multifocal intraocular lens implantation

*CHIAM P, CHAN J, KASABY HE, AGGARWAL RK*
*Eye, Southend, London*

**Purpose** To evaluate the visual outcome and patient satisfaction of a new multifocal intraocular lens (IOL) implantation, the AcrySof ReSTOR SN60D3 (Alcon) for cataract patients with presbyopia.

**Methods** Fifty-eyes of 28 patients had implantation of the multifocal posterior chamber IOL after cataract extraction. The patient selection method, preoperative evaluation, surgical technique, postoperative visual and refractive outcomes, and complications were assessed retrospectively. Patient satisfaction and quality of vision were assessed using a standardized questionnaire over the phone 3 weeks postoperatively. Patients who had more than 1 D of corneal astigmatism preoperatively had limbal relaxing incision during phacoemulsification. All operations were performed by 2 surgeons without complication. The average preoperative cylinder was 0.74 D. The preoperative refractive error ranged from -4.75 D to +2.75 D. Corneal incision for phacoemulsification was performed on the axis of steepest meridian.

**Results** All eyes achieved uncorrected distance acuity of 6/12 or better, with 86% achieving at least 6/7.5. 94% achieved an uncorrected near acuity of N5 or better. All patients were satisfied with the quality of their vision postoperatively except for 1 patient who complained of halos at night.

**Conclusion** The AcrySof ReSTOR IOL implantation has predictably good uncorrected distance and near acuities postoperatively. Patient satisfaction was high and complaints of undesirable visual effect was low.

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## Capsular shrinkage after phacoemulsification and implant surgery

*KULJACA Z, DZAKOVIC N, RADOVIC N, IGNJATOVIC Z, BEKO M*
*Milos Clinic - Eye Hospital, Belgrade*

**Purpose** To analyse the capsular shrinkage (CS), development and risk factors, after phacoemulsification (PE) and implant surgery ( IOL ).

**Methods** A retrospective case - note review study in 2000 patient ( 2112 ) eyes, undergoing PE with IOL and followed postoperatively up to 24 months, was carried out. Statistical analyses were performed on categoric variables and any difference showing p vallue of less than 0.05, was considered statistically significant.

**Results** Overall, in 30 eyes ( 1,42 % ), CS with contraction of anterior capsule opening and IOL dislocation, was found. Mean age group at the time of surgery was 76.8 yrs in male and 71.9 yrs in female. Secondary cataract developed by 6 months time in 14 patients, in 6 - 12 months in 11 patients and in 12 - 24 months time in 5 patients, postoperatively.CS risk factors found were: glaucoma ( 12 patients ), pseudoexfoliative syndrome ( 2 patients ), diabetes ( 2 patients ), myopia ( 2 patients ), myopia and glaucoma ( 1 patient ) and diabetes and glaucoma ( 1 patient ). In 9 eyes ( 30 % ), no risk factors were indentified at all.Among preoperative risk factors, glaucoma expressed significantly higher rate of incidence, compared to others ( t = 0,956 ) and CS showed up more frequently in females ( t = 0,693 ). No significant difference in time of CS appearance, compared to risk factors, was found, at all.

**Conclusion** Preexisting glaucoma disease, particulary in female patients, could be related with CS after PE with IOL, more than the other risk factors.

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## Metabolic differences in rat lens regions after UV-B irradiation analysed by HR–MAS 1H NMR spectroscopy

*TESSEM MB (1), BATHEN FT (2), LÖFGREN S (3), MODY V (3), MEYER L (3), DONG X (3), SÖDERBERG PG (3), MIDELFART A (1)*
*(1) Ophthalmology, Faculty of Medicine, Norwegian University of Science and Technology, Trondheim*
*(2) Circulation and Medical Imaging, Faculty of Medicine, Norwegian University of Science and Technology, Trondheim*
*(3) St. Erik's Eye Hospital, Karolinska Institutet, Stockholm*

**Purpose** To investigate metabolism in different regions (anterior, posterior, nucleus and equatorial) of the UV-B irradiated rat lens analysed by HR-MAS 1H NMR spectroscopy and multivariate analysis.

**Methods** One eye of thirteen Albino Sprague-Dawley rats was exposed to UV-B radiation for 15 minutes (300 nm, 7.5 kJ/m2) under anaesthesia in vivo. The other eye was used as control. Each rat was killed by CO2 asphyxiation 7 days after exposure, and the lenses were dissected from enucleated eyes. The lenses were cored by a trephine (2.5 mm bore) and sliced in four regions (anterior, posterior, nucleus and equatorial) by a homemade slicing machine. Samples for NMR analysis were made by pooling equivalent regions from four lenses. Analysis was performed by HR–MAS 1H NMR spectroscopy on a Bruker Avance DRX600 spectrometer (14.1 T), and the metabolic profile of the samples was statistically analysed by Principal Component Analysis (PCA).

**Results** More than 25 metabolites were detected in the spectra of each rat lens region. The PCA separated the samples from different regions of the lens into distinct clusters both within exposed and unexposed samples. Distinct clusters were in addition found between all the exposed and unexposed samples of the lens.

**Conclusion** Marked differences in metabolism were observed between the different regions of the lens both within and between the exposed and unexposed groups.

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## Lens optical quality: the area under the modulation transfer function correlates with visual acuity

*SHAMS PN (1), DAVIES NP (1), WEALE RA (2)*
*(1) Ophthalmology. Chelsea and Westminster Hospital, London*
*(2) Ophthalmology. University College London Hospital, London*

**Purpose** The OQUAL™ is a device that attaches to a slit lamp and projects a series of gratings of increasing periodicity into the eye. The 4th Purkinje image is captured with a digital camera. The resulting image is analysed to derive the modulation transfer function (MTF) of the lens as a measure of its optical quality.

**Methods** In this study 33 patients with increasing grades of cataract and no other pathology were imaged and their visual acuity measured. The MTF was calculated from the grating image using a Fourier transform method described previously (EVER 2003) and the total area under the MTF calculated. This was then compared with the visual acuity.

**Results** There is a significant correlation of area under the MTF with visual acuity measured as a Snellen fraction. The MTF area decreased linearly as a function of decreasing Snellen fraction as cataract worsened. The correlation coefficient was 0.73 giving r2= 0.58 ($p<0.0001$).

**Conclusion** The area under the MTF as measured using the OQUAL gives an independent measure of lens quality that correlates with the visual acuity. In cases of reduced visual acuity due to both cataract and macular disease, the OQUAL can be used to separate the effect of cataract on the visual acuity, to ascertain whether cataract surgery would be likely to be of benefit.

## ▪ 349 / 3453
## 3D images of cataract surgery: a new telemedicine and didactic tool

*LIBONDI T (1, 2, 3), CRICELLI R (1), CAPUTO S (1), ZEPPA L (4), GIACOIA A (1), COLONNA G (2)*
*(1) Dept of Ophthalmol. II Univ, Napoli*
*(2) CRISCEB (Center for Res. in Comp.Sci. and Biotech.) II Univ, Napoli*
*(3) Medical Services sas, Napoli*
*(4) UOC Ocul. Osp S. Giuseppe Moscati, Avellino*

**Purpose** The importance of stereo viewing in microscopic ophthalmic surgery is well known. Surgical images are nowadays bidimensional, lacking the third dimension. We wanted to test the possibility to obtain and store stereo images obtained at the microscope during cataract surgery.

**Methods** A Zeiss OPMI 1 surgical ophthalmic microscope was equipped with two C-mount adaptors in such a way that each of them was able to capture left and right images of the surgical field as seen by the surgeons. Two Olympus Camedia C-4040 Zoom digital cameras were used to simultaneously acquire left and right images. They were stored in JPEG format in a dedicated workstation, and thereafter processed and rendered by using Stereographics-3D (TM) software and Crystall Eyes 3 goggles on a high definition monitor.

**Results** High definition stereoimages of the anterior segment of the eye as seen at the microscope were acquired during routine surgery for cataract (phacoemulsification and IOL implantation). The main problems encountered with our setting were proper focusing and exact alignment of the two different images. The approx. size of a single left or right JPEG image was about 1 Mb, thus permitting a rather easily storage and forward through the net. By viewing them with stereo rendering, the spatial relationships and the relative depth of the different structures could be clearly appreciated, as opposed to the single left and right images when seen separately.

**Conclusion** It was possible to take, store, send and render 3D images as taken at the microscope during a phacoemulsification procedure for cataract. The addition of stereopsis added reality to the images, as compared to the single flat bidimensional image.

## ▪ 350 / 3455
## Variation in the theatre team and its impact on the outcome of complicated cases

*BAYLIS OJ, ADAMS W, FRASER SG*
*Sunderland Eye Infirmary, Sunderland*

**Purpose** The outcome of complicated (i.e. posterior capsule rupture) cataract surgery partly depends on how effectively the complication is dealt with. Rapid assessment and efficient management of the problem can make all the difference. This study was designed to investigate how the variations in experience of non-medical personnel in theatre could affect the outcome of a complicated case. For the purpose of the study we defined a good outcome as one in which the patient did not have to return to theatre for a further corrective procedure (e.g. further vitrectomy).

**Methods** 100 complicated cataract cases were identified. For each case, the experience of the scrub nurse and the cumulative experience of the nursing team present were recorded. Any procedures that the patient underwent following the initial surgery were also recorded.

**Results** 7 out of 50 (14%) complicated cases involving scrub nurses with 10 or more years experience required further procedures. 10 out of 32 cases (31%) with scrub nurses with 7 or less years experience required further procedures. In the 'more experienced' cohort for cumulative experience, 8 further procedures were performed. In the 'less experienced' cohort, 14 were done.

**Conclusion** These results suggest that when cataract surgery is complicated by posterior capsule rupture, fewer patients need corrective surgery later when more experienced non-medical staff are present in theatre. Case mix for both groups was similar suggesting that this could be due to greater experience allowing a more rapid and effective response by the non-medical team, permitting the surgeon to rescue the primary procedure e.g. by completing the anterior vitrectomy or placing a sulcus lens. As far as we are aware this is the first time this has relationship has been found.

## ▪ 351 / 3456
## Intraoperative breakage of the mushroom manipulator tip during phacoemulsification

*PELOSINI L, RICHARDSON EC, GOEL R, HUGKULSTONE CE*
*Queen Mary's Hospital, Sidcup*

**Purpose** To report a series of three cases of breakage of mushroom nucleus manipulators during phacoemulsification performed by two different surgeons in the same Unit.

**Methods** The three reported incidents occurred to two different surgeons over a period of three months. Case 1: the mushroom manipulator tip was missing at the end of the phacoemulsification, the capsular bag was filled with viscoelastic and the broken tip was retrieved with capsulorhexis forceps.Case 2: at the end of a successfull phacoemulsification the second instrument was removed from the eye and the mushroom head broke off the stem, remaining embedded on the side port. The fragment was dislodged with viscoelastic and removed using suction of a lacrimal cannula through the main wound. Case 3: the mushroom tip was noticed to be floating free in the capsular bag during the segments removal and was removed by gentle aspiration with a lacrimal cannula.

**Results** In all cases the breakage of the manipulator tip was detected at the end of nucleus phacoemulsification, the fragment was successfully retrieved and patients did not suffer any adverse effect. The examination of the instruments revealed significant wear around the stem, decreased thickness of the material and numerous surface marks by abrasive action.

**Conclusion** Intraoperative breakage of instruments during phacoemulsification may lead to severe complications, this is the first series of intraoperative macroscopic breakage of the same type of nucleus manipulator without ocular damage.We would recommend care to avoid inadvertent contact between instruments within the anterior chamber during phacoemulsification.

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## Effect of Sodium Hyaluronate (Ophthalin®) and Hydroxypropylmethylcellulose (HPMC-Ophtal®) on Corneal Endothelium, Central Corneal Thickness, and Intraocular Pressure after Phacoemulsification

*VOROS GM, RESSINIOTIS T, RAY-CHAUDHURY N, SUTHERLAND S, MITCHELL K, FIGUEIREDO FF*
*Royal Victoria Infirmary, University of Newcastle upon Tyne, United Kingdom., Newcastle upon Tyne*

**Purpose** To prospectively evaluate the effects of 2% hydroxypropyl-methylcellulose (HPMC-Ophtal®) and sodium hyaluronate 1% (Ophthalin®) on intraocular pressure, corneal thickness and endothelial cell loss in small incision cataract surgery with implant.

**Methods** 110 patients undergoing routine phacoemulsification with implant received either 2% hydroxypropylmethylcellulose or sodium hyaluronate 1% as ophthalmic viscosurgical device. Pre- and post-operative slit lamp examination, intraocular pressure measurement (pre-operatively and at 1-4 hours, 1 day and 7 days post-operatively), ultrasonic pachymetry (pre-operatively and at 1 week, 4-6 weeks, and 12 weeks post-operatively) and corneal endothelial cell count (pre-operatively and 12 weeks post-operatively) were performed. Data was analysed using two way analysis of variance.

**Results** Intraocular pressure was significantly lower in the Ophthalin® group at 1 day post-operatively, while no significant difference was found between the two groups on the 1-4 hours and 7 days examination. Post-operative central corneal thickness was not significantly different between the two groups. The mean cell density demonstrated a significant fall of 11.76% for Ophthalin® and 4.27% for HPMC-Ophtal® at twelve weeks post-operatively, the difference between the two being significant (p=0.009).

**Conclusion** 2% hydroxypropylmethylcellulose, compared with sodium hyaluronate 1%, is superior in protecting the corneal endothelial cells, has the same effect on central corneal thickness, and is associated with slightly higher intraocular pressure one day post-operatively.

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## Tridimensional representation of KID syndrome mutations in GJB2 gene: from structure to function

*MENNECIER G (1), GICQUEL JJ (1, 2), DIGHIERO PLD (1), MESNIL M (2)*
*(1) Ophthalmology Poitiers University Hospital, Poitiers*
*(2) Equipe Interactions et Communications Cellulaires, CNRS UMR 6187, Poitiers*

**Purpose** KID (Keratosis Ichthyosis and Deafness) Syndrome is characterized by progressive visual loss due to limbal stem cells deficiency. It is caused by mutations in the GJB2 gene which encodes the gap junction protein connexin 26 (Cx26). We will try to apprehend the functionality of 8 of these mutations, with a three-dimensional computerized model.

**Methods** Eight mutations of the GBJ2: Gly12Arg (G12R), Ser17Phe (S17F), Asp50Asn (D50N), Asn54Lys (N54K), Gly59Ala (G59A), Asp66His (D66H), Arg75Try (N75W) and Arg75Glu (N75Q) were analyzed. The 8 mutated Connexins were modelized in 3 dimension by the computer. We compared our models to functional data obtained in vitro on HeLa cells.

**Results** The eight mutations are located in the highly conserved sequence of the extracellular domain 1 and cytoplasmic loop of connexin 26. These mutations disrupt gap junctionnal communication. Other GJB2 mutations that do not concern conserved amino acids, may not modify the function of the connexin and keep its normal physiology.

**Conclusion** Mutations observed in KID syndrome affect important conserved amino acids present in the others human connexins. Cell-cell communication mediated by connexins is crucial to the regulation of cell growth, differentiation, and development in the cornea.

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## Fine mapping of a autosomal dominant cone-rod dystrophy (CORD5) ) to a 2.61 Mb region on 17p13.1-p13.2

*KÖHN L (1), MOHARER J (1), HARALDSSON S (1), SANDGREN O (2), GOLOVLEVA I (1)*
*(1) Medical and Clinical Genetics/Medical Biosciences, Umeå*
*(2) Ophthalmology/Clinical Biosciences, Umeå*

**Purpose** Cone-rod dystrophy, CORD5, is an autosomal dominant retinal disorder characterized by degeneration of cone photoreceptor cells, causing an impaired visual acuity, sensitivity for light and defective color vision. The CORD5 locus has previously been mapped to chromosome 17p12-p13 located in a region of 25 cM between the markers D17S926/D17S849 and D17S804/D17S945. The purpose of this study was to refine the region containing the disease gene.

**Methods** Fine mapping of the region was done by using twelve 2 cM microsatellite markers mapped to 17p13 in two families with autosomal dominant CORD5 orginating from Jämtland. Subsequent evaluation of the data was done with linkage and haplotype analysis.

**Results** Two-point linkage analysis in the TAD-A family gave a maximum lod score of 6.51 at the marker D17S938 with significant lod scores between markers D17S1828 and D17S945, indicating the disease interval. In TAD-F family significant evidence of linkage was demonstrated at the markers D17S678, D17S938, D17S1881, D17S720 and D17S1844. Maximum lod score of 4.54 was obtained at marker D17S678. Reconstructed haplotypes in both families confirmed disease segregation with markers, D17S678, D17S938, D17S1881, D17S720 and D17S1844.

**Conclusion** The CORD5 locus has been narrowed down from 25 cM to approximately 3.77 cM (2.61 Mb). The reduced interval contains several candidate genes and mutation analysis is in progress.

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## No association between primary open angle glaucoma and WAF1 gene in Caucasians

*RESSINIOTIS T (1, 2), VOROS GM (1), GRIFFITHS PG (1), KEERS SM (2), CHINNERY PF (2), BIRCH M (1)*
*(1) Department of Ophthalmology, Royal Victoria Infirmary, Newcastle upon Tyne*
*(2) Department of Neurology, The Medical School, The University of Newcastle upon Tyne, Newcastle upon Tyne*

**Purpose** Primary open angle glaucoma (POAG) is considered to be a neurodegenerative optic neuropathy, in which cell death occurs by apoptosis. WAF1 (p21), is an important protective component of the apoptotic pathway, regulating cellular arrest. An unstable or altered p21 protein could modify the cellular response to genomic injury and abolish the effect of p21. A previous study on a Chinese cohort suggested that the p21 codon 31 polymorphism may alter the state of apoptosis in glaucomatous optic neuropathy. The aim of this study was to test the hypothesis that a p21 codon 31 polymorphism is associated with POAG on a Caucasian cohort.

**Methods** 140 POAG patients and a control group of 73 healthy subjects, all Caucasians, were included in the study. Genomic DNA was amplified by PCR, followed by enzymatic restriction fragment length polymorphism technique (PCR-RFLP). Patients and controls were genotyped for a single nucleotide polymorphism (C/A transversion) in the third base of codon 31 of p21, which leads to a serine (Ser)/arginine (Arg) substitution.

**Results** The distribution of the genotypes in the POAG patients showed 128 (91.4%) Ser homozygotes, 10 (7.1%) Ser/Arg heterozygotes and 2 (1.5%) Arg homozygotes. In the control cohort, there were 61 (83.6%) Ser homozygotes and 12 (16.4%) Ser/Arg heterozygotes. No Arg homozygotes were present amongst the control group. Both the allelic and genotypic frequencies of the Ser or Arg residues at codon 31 were not significantly different between POAG patients and controls (Fisher's exact test, P = 0.20 for alleles and P=0.0561 for genotypes).

**Conclusion** This study suggests that the p21 codon 31 polymorphism does not contribute to the risk of POAG in the Caucasian population.

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## Aey12, a new mutation affecting the early eye development

*PUK O (1), LÖSTER J (1), BECKERS J (2), GRAW J (1)*
*(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 Experimental Genetics, Neuherberg*

**Purpose** Aim of the study was the morphological and genetical characterization of Aey12, a new dominant mouse mutant suffering from microphthalmia, lens and corneal opacities.

**Methods** Aey12 was established as a new mutant line in the mouse. A genome wide linkage analysis was performed using micro-satellite markers. Histological analysis and in-situ hybridizations were performed at the stage E12.5. An expression array was undertaken at E10.5.

**Results** Aey12 (abnormality of the eye) is a new dominant mouse mutant line which was recovered in an ENU mutagenesis program at the GSF. Aey12 animals are primarily characterized by small eyes with cornea opacities and lenses with cataracts and vacuoles. Both, hetrozygotes and homozygotes, are viable and fully fertile. As major histological defect, the block of primary lens fibre differentiation after the closing of the lens vesicle and therefore the stop of lens development in this stage were detected. Genome-wide linkage analysis mapped the Aey12 mutation on mouse chromosome 10 between the markers D10Mit123 and D10Mit206. Expression arrays revealed an influence of this mutation on several genes including Pex3 and Deadc1 of the critical interval on chromosome 10. These two genes are arranged in a head-to-head orientation with a spacer of 135 bp. Their coding- and promoter regions are not affected. However, the reduced expression in the eye indicates an influence of the Aey12 mutation on the regulation of Pex3 and Deadc1.

**Conclusion** The new mouse mutant Aey12 is characterized by an early block of the eye development. Mapping data, gene expression arrays and in-situ hybridizations point to an altered regulation of the genes Pex3 and Deadc1 as the causative event in the Aey12 mutant. The mutation in Aey12 remains to be identified.

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## Ocular components data in young adults and their correlation with the refractive error

*QUEIROS A, GONZALEZ-MEIJOME J, JORGE J, FRANCO S*
*Physics (Optometry), Braga*

**Purpose** The aim was to investigate the correlation between the refractive error and ocular components in a group of young adults.

**Methods** The exams were performed on 119 young adults (71.4% females) with a mean age of 23.2 ± 2.37 years. Axial ocular dimensions, including axial length (AL), anterior chamber depth (ACD), lens thickness (LT), and vitreous chamber depth (VCD) were measured using an A-scan ultrasound device. Corneal radius (CR) and eccentricity (CE) was measured with an autokeratometer.The results obtained by the subjective distant refractive method with cycloplegia were used in the analysis and the refractive values were converted into spherical equivalents (SE) for some analysis. Myopia was defined as SE<=-0.50D, emmetropia as SE >-0.50D and <+0.50D and hyperopia as SE >=+0.50D.

**Results** The incidence of refractive errors was 26.9% miopes, 31.9% emmetropes and 41.2% hyperopes. The refractive error of the sample, ranged from −9.75D to +2.00D, was −0.357 ±1.60D (mean±SD). The maximum amount of astigmatism was −2.50D.The AL, ACD, LT, VCD, CR, and CE values were 23.49±0.99mm (mean±SD), 3.59±0.26mm, 3.68±0.16mm, 16.21±0.92mm, 7.81±0.27mm and 0.49±0.11, respectively. There were found statistically significant differences ($p<0.001$) between the three groups of refractive error (M, E and H) for the AL, ACD, and VCD. For LT, CR, and CE there are no significant differences.A statistically significant ($p<0.001$) correlation between the refractive error and the ACD, VCD, and AL was found.

**Conclusion** The results show an incidence of refractive error similar to those obtained in other countries. The ACD, VCD, and AL are the ocular components with the highest influence in the refractive error state.

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## An automated system of measuring decentration of anterior segment structures from geometric central axis

*ASLAM T M (1), GUPTA A (1), ROSE C (2), CHARLES S J (1)*
*(1) Royal Eye Hospital, Manchester*
*(2) Department imaging science and biomedical engineering, Manchester University, Manchester*

**Purpose** To develop and test a system of automated analysis of amount of decentration of anterior segment structures from a geometric axis defined either by pupil or limbal landmarks. The system should be freely available and accessible for use without specialised equipment.

**Methods** The system consisted of a computer program developed using the Matlab programming platform. Standard digital images of anterior segments were used by the program to make measurements. The program code was also compiled for use on machines without the Matlab platform.The geometric central axis is computed based on either limbal or pupil structure, as defined by the user. The object to be measured is also defined by the user.This object may be the pupil itself (to be measured relative to limbus) , crystalline lens, intraocular lens, corneal opacity, posterior capsule opacification or other anterior chamber body. The amount of decentration and angle of decentration of the object is calculated.The system was tested for reliabiltiy and validity.

**Results** The compiled system is fully functional on PCs running Windows XP. It is easily adaptable to assess decentration of a variety of structures using standard digital images. The reliability of the system was found to be high. Ample evidence for validity of the program was demonstrated.

**Conclusion** This paper presents as system of analysis of decentration of anterior segment bodies that requires no specialised equipment.The program and necessary files can be installed using the supplied software. The system is shown to be extremely versatile , valid and reliable. It may be used clinically to track progress of individual patients or as an objective measure for experimental studies.

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## Prospective Study on the Prevalence of Refractive Conditions in the North of Portugal

*QUEIROS A (1), JORGE J (1), GONZALEZ-MEIJOME J (1), FERNANDES P (1), ALMEIDA JB (1), PARAFITA MA (2)*
*(1) Physics (Optometry), Braga*
*(2) Surgery (Ophthalmology), Santiago de Compostela*

**Purpose** The purpose of this study was to report the first estimations on the prevalence of refractive conditions in the Portuguese population

**Methods** The files of an optometrist consultant in the north of Portugal were reviewed, and the report of 4288 patients who attended the clinic at least once is given; only the first visit of each patient was considered. Results relate to spherical equivalent value under non-cycloplegic conditions. Values of non-cycloplegic refraction from the right eye were analyzed to estimate the prevalence of refractive conditions as a function of age and gender. The prevalence of anisometropia and presbyopic correction as a function of age and gender were also investigated

**Results** A total of 4288 patients with a mean age of 40.08 ± 18.75 (mean ± SD). Mean spherical equivalent refractive error was - 0.29 ± 2.01 D, and no statistically significant gender differences were found. A total of 29.8% of the sample had myopia, 45% had emmetropia and 25.2% had hyperopia. Young adults ranging from 20 to 35 years of age presented the highest prevalence of myopia. Conversely, the peak of hyperopic condition was for the oldest population. There were not statistically significantly differences between presbyopic correction between males and females and both followed the classical models of presbyopic correction as a function of age.

**Conclusion** The prevalence of refractive conditions has been established for a representative group of the Portuguese population. Important refractive changes were evidenced in the fifth decade of life, comprising an increase in the prevalence of hyperopia along with a shift in the amount of astigmatism

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## A Quick Look at the Lid Surgeries We Do

*RAVINDRANATHAN R, TAHHAN M*
*Ophthalmology, West Bromwich*

**Purpose** Introduction: The types of surgery and procedures done on the lid are many for the various conditions like entropion, ectropion, dermatochalasis, papilloma and basal cell carcinoma. We thought it would be interesting to look into our lid surgeries and to note the results.Objectives: To study the outcome of various lid surgeries done at Sandwell Hospital between January 2001 and December 2002 on patients with Entropion, Ectropion and Dermatochalasis.

**Methods** Setting: Sandwell General Hospital in West Midlands, United KingdomMethodology: Retrospective analysis of case notes- 41 cases. We obtained the names of the patients from the theatre register and analysed it retrospectively. The following data was collected : patient details, diagnosis, reason for surgery, type of surgery, grade of surgeon, complications, surgical outcome, recurrence of symptoms and repeat operations. The number of male and female patients were 22 and 19 respectively.

**Results** Results: Out of 41 cases, 29 had good surgical outcome. Out of 12 cases which had a poor outcome, 6 were previous recurrences. Poor outcomes included recurrences, presence of notch and residual condition. The most common case operated was Entropion. Quickert's procedure was the most commonly done operation.

**Conclusion** Conclusion: According to our research, it was found that patients with medial canthal laxity had poorer results. It was decided that such patients and those with a recurrence should be sent to the occuloplastic surgeons in the future.

## ▪ 361
## Avulsion of the eyeball - a rare injury in childhood (case report)

*VAMOS R, FARKAS A, SALACZ G*
*Semmelweis University, Department of Ophthalmology, Budapest*

**Purpose** To present a rare clinical case of avulsion of the eyeball and discuss possible mechanisms of this serious injury.

**Methods** A 9 years old boy suffered a grievous hurt to his right eye caused by a door-handle while the door was being shut. Ophthalmological and neurological examinations and computer tomography of the skull and orbit were carried out.

**Results** The optic nerve ruptured and the eyeball left the orbit. No neurological pathological alterations were found clinically. CT examination showed neighter intracranial pathology nore fractures of the orbital bones or skull. There was an inhomogenous soft tissue in the right orbit and the optic nerve sheath was thickened.

**Conclusion** Indoor activities amoung children may cause dangerous trauma to the eye and orbital soft tissues.Possible mechanisms of avulsion include foreward luxation and forced rotation of the eyeball.

## ▪ 362 / 2112
## Homozygote cytokeratin 12 mutation in a Danish family with Meesmann's dystrophy

*NIELSEN KN (1), ORNTOFT TFO (2), HJORTDAL JH (1), EHLERS NE (1)*
*(1) Department of Ophthalmology, Aarhus*
*(2) Molecular Diagnostic Laboratory, Aarhus*

**Purpose** Meesmann's dystrophy was first described by Meesmann & Wilke (1939) as myriads of microcysts in the corneal epithelium seen by slit-lamp microscopy. Mutations in the cytokeratin 3 or cytokeratin 12 genes are considered to be responsible for the dystrophy (Nishida 1997, Irvine 1997). We here describe such a mutation in a small Danish family of a mother and her daughter.

**Methods** Clinically the mother showed microcysts in the entire epithelium. By contrast, the daughter presented only discrete cystic changes. Blood samples were collected and DNA was isolated. All 8 exons were sequenced on the 3100 Genetic Analyzer. Primers for exon 1a were: 5'-ccatcttgcagcctatataagtttagc-3' and 5'-cgagagaatacctagagagccacc-3'. DNA sequence analysis was performed with the Sequencher software.

**Results** DNA sequencing of the 1.9 kb coding cytokeratin 12 gene showed a 67C→T mutation in the family. The missense mutation gives rise to amino acid substitution Pro15Ser. The mother was homozygotic 67C→T and the daughter was heterozygotic 67C→T. Both parents to the mother were deceased and the origin to the homozygotic mutation could not be established.

**Conclusion** This mutation in the 5'-end of the gene contributes to the characterization of cytokeratin 12 gene in relation to the dystrophy. Most mutations are identified in a hotspot region (position 410-451) but this new mutation causes an amino acid substitution at the N-terminal of this structural protein.Meesmann's dystrophy is described as an autosomal dominant disorder and so a single mutation in one of the alleles is adequate to cause the disease. These data represent a patient who is homozygote of this rare mutation and who shows more severe changes than the heterozygotic daughter.

## ▪ 363 / 2115
## Study of Primary Mutation and Clinical Features of Leber's Hereditary Optic Neuropathy in Chinese Patients

*GUO X, WANG Y, JIA X*
*Molecular Genetics, Zhonshan Ophthalmic Centre, Guangzhou*

**Purpose** To study primary mutation and clinical features in Chinese patients with Leber's hereditary optic neuropathy (LHON).

**Methods** Three primary mtDNA mutations (3460A,11778A and 14484C) of 156 Chinese LHON patients were detected by MSP-PCR, HA-SSCP, RFLP and DNA sequence. The clinical features were analyzed by retrospective study.

**Results** Of the 110 probands, the 11778A mutation was found in 100 probands (90.9%), the 3460A in 2 (1.8%), and the 14484C in 8 (7.3%).16 of 250 eyes with the 11778A mutation recovered a mean final visual acuity of 0.03, whereas 28 of 56 eyes with the 14484C recovered a mean final visual acuity of 0.8.

**Conclusion** In Chinese LHON patients, the 11778A mutation are common. The visual acuity of visual recovery with 14484C were better than one with 11778A mutation.

## ▪ 364 / 3115
## To ascertain the prevalence of poor vision which exists in boys at a prestigious school in a developing country (Trinidad)

*DWARIKA D (1), BHOLA RM (2), SEEMONGAL DASS R (3), EMAMALIE S (2)*
*(1) Birmingham and Midland Eye Centre, Birmingham*
*(2) Academic Unit of Ophthalmology and Orthoptics, The University of Sheffield, Royal Hallamshire Hospital, Sheffield, Sheffield*
*(3) The Eric Williams Medical Sciences Complex, Trinidad*

**Purpose** This study aims to ascertain the prevalence of poor vision which exists in boys in a prestigious school in a developing country (Trinidad).

**Methods** The study included two parts: a short observer administered questionnaire and an assessment of visual acuity. Monocular visual acuities were assessed using imperial standard Snellen charts from a distance of 20 feet. Visual acuities were performed with glasses, unaided, and pinhole. Glasses were neutralized and results recorded.

**Results** A total of 334 students ranging in age from 11 to 18 were tested. Seventy students failed to achieve the required visual acuity in one or both eyes (20.96%). One hundred and seventy students never had an eye test (51%). Of the one hundred and sixty four who did have an eye test, 46 had a refractive error needing correction. Eighteen were myopic and the remaining 28 had myopic astigmatism.

**Conclusion** More that 50% never had a formal eye examination and 1 in 5 students failed to achieve a visual acuity of 20/25. This result emphasizes the need for formal vision screening in schools in Trinidad.

## ▪ 365 / 3116
## Ocular complications and evaluation of quality of life in patients after Stevens-Johnson and Lyell syndromes

*GUEUDRY J (1), BINAGHI M (2), SOUBRANE G (2), ROUJEAU J-C (3), BRASSEUR G (1), MURAINE M (1)*
*(1) Ophthalmology, Rouen*
*(2) Ophthalmology, Créteil*
*(3) Dermatology, Créteil*

**Purpose** Toxic epidermal necrolysis (TEN) and Stevens-Johnson syndrome (SJS) are rare, life-threatening, drug-induced mucocutaneous reactions. The purpose of these present report is to describe the ocular sequelae and to quantify their impact on quality of life.

**Methods** We retrospectively reviewed the medical records of patients with Stevens-Johnson and Lyell syndromes seen between 1994 and 2002 at the university hospital of Creteil. Patients underwent a recent ophthalmologic examination and completed the OSDI (ocular surface disease index). It measure dry eye disease severity and effect on vision related function. It is assessed on a scale of 0 to 100, with higher scores representing greater disability.

**Results** One hundred and sixty five patients were identified. Thirty two patients (19%) died during their hospitalisation, 17 patients (10%) died secondarily, 11 patients were excluded because of their poor compliance, finally 42 patients were non completers.OSDI of 66 patients were analysed. Mean age was 47 years. Mean follow up was 7 years. Sixty five percent of patients had dry eye symptoms, mean OSDI score was 31. Fifty one patients underwent clinical examinations. Best corrected visual acuity was 6/10 or better in 32/44 (96%) patients. Lid abnormalities were observed in 8/51 (16%) patients, conjunctival abnormalities in 16/51 (29%), corneal complications in 15/51 (29%) and sicca eyes syndrome in 30/51(59%).

**Conclusion** We report here the most important series of patients with SJS syndrome and TEN. In contrast with other smallest and rare series, only few patients (7%) have severe ocular complications with very poor quality of life.

## ▪ 366 / 3117
## Missed metal Foreign Bodies

*SKINNER KC, CHRONI FC, MERRICK CD, GREGORY PT*
*Conquest hospital, St-Leonards-on-Sea*

**Purpose** To emphasise the importance of history taking where a possible metal foreign body is suspected. The importance of x rays in these case and their suitability as a screening process.

**Methods** Case Series

**Results** Three patients all presenting with metal foreign bodies detected by means other then direct observation.One: attended A and E in 2000. History, hammering metal nail. A and E SHO noticed possible metal foreign body embedded in sclera. Patient seen by on call eye SHO no foreign body noted but large sub conjunctival haemorrhage. Patient subsequently DNA all follow up appointments. 5 years later attended for MRI scan due to possible fits. X ray of orbits done as patient reported past history metal FB. Xray revealed presence metal in anterior aspect of globe.Two: attended A and E in 2005 after hammering nail. Three days later developed very painful red eye. A and E SHO could not see any FB but could see small scar on cornea. Xray done and metal FB noted in anterior segement. Eye SHO saw patient and noted large FB embedded in temporal aspect of iris.Three: attended eye clinic for cataract. Staff grade saw patient and noticed abnormal shaped optic disc. Seen by consultant who noted possible metal FB embedded in optic disc, in fact later old notes revealed probably been there since 1976.

**Conclusion** IOFB of metal composition leads to long term complications such as siderosis etc. It is therefore very important not to miss these when seeing a patient. One indicator to the high possibility of IOFB is the history that the patient gives, e.g hammering metal on metal. In these cases a very thorough examination must be performed and if no FB found then Xray considered. As MRI is becoming more used it is especially important not to miss metal IOFB.

## ▪ 367 / 3118
## OPHTEC Iris Reconstruction Lens in Ocular Trauma

*KULJACA Z (1), IGNJATOVIC Z (1), DZAKOVIC N (1), PAVLOVIC S (2, 1)*
*(1) Milos Clinic, Belgrade*
*(2) University of Giessen,Dpt. of Ophthalmology, Giessen*

**Purpose** To see how penetrating eye injury in young patient with visual and cosmetic sequels can be repaired with satisfactory results concerning visual acuity as well as good looking.

**Methods** Young female, aged 17, presented with penetrating injury of her left eye that was primary repaired two weeks ago. She was injured with glass in car crash and had intumescent traumatic cataract, vitreous hemorrhage and complete avulsion of the iris. In the first step pp lensectomy and vitrecomy was performed. Due to peripheral retinal tears silicon oil tamponade was necessary. After three months in second step surgery silicon oil was removed and Ophtec aniridia IOL was placed in sulcus using the remaining parts of the anterior capsule.

**Results** After four months the visual acuity was 0,7 and the retina is attached. There is no glare or photophobia and both eyes look the same in everyday life.

**Conclusion** After major and disfiguring ocular trauma it is important to repair both vision and appearance whenever possible in order to fully satisfy the patient.

## ▪ 368
## Ciliary epithelial cells estimation during experimental diabetes

*MACHOWICZ-MATEJKO E*
*I-st Eye Hospital and T. Krwawicz Chair of Ophthalmology Medical Academy, Lublin*

**Purpose** Morphological and ultrastructural assessment of the ciliary epithelium during alloxan – induced diabetes.

**Methods** White New Zealand male rabbits were used. Experimental diabetes was induced with 10% Alloxan solution injected i.v. After 3 and 6 weeks, 3 and 6 months animals were sacrificed, 5x6 mm fragments of the ciliary body pars plicata were removed, fixed and processed for light and electron microscopy. Basement membrane thickness and size of the nonpigmented and pigmented epithelia cells and nuclei was measured and analyzed statistically using Kruskal – Wallis and Mann – Whitney tests.

**Results** Nonpigmented ciliary epithelium basement membrane thickening was observed, which increased with the duration of the experimental diabetes. Also the epithelial cell size was increasing during the experiment, in contrast to the size of nuclei, which was decreasing with time. Transmission electron microscopy revealed the damaged of epithelial tight junctions. Cells nuclei were pycnotic, mitochondria were swollen as well as the cisterns of endoplasmic reticulum. In the cell cytoplasm glycogen granules were observed. Pericapillary fibrosis was also observed.

**Conclusion** Morphological changes observed in the ciliary epithelium and capillaries suggest damage to the blood-aqueous barrier in the course of experimental diabetes in rabbits.

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## Scleritis and Bilateral Exudative Retinal Detachments as a First Presentation of Metastatic Carcinoma

*WRIDE NK (1), COTTRELL DG (1), BIRCHALL D (2), PANDIT RJ (1)*
*(1) Department of Ophthalmology, Royal Victoria Infirmary, Newcastle-upon-Tyne*
*(2) Department of Radiology, Royal Victoria Infirmary, Newcastle-upon-Tyne*

**Purpose** To report a rare case of metastatic carcinoma masquerading as scleritis and to underline the need to re-evalute the management and diagnosis of patients with atypical presentations.

**Methods** A 73 year old man presented to our department with a 4 month history of increasing ocular pain, reduced vision and redness of the left eye. On examination he had a severe bilateral anterior scleritis with bilateral exudative retinal detachments. He had reported weight loss but had no other symptoms.

**Results** He responded to high dose steroids which settled the pain and redness. However, the retinal detachments persisted, becoming total on the left side. His inflammatory markers remained elevated and despite thorough investigation, no systemic cause could be found. Ocular ultrasound revealed a mass in the retro-orbital space close to the left optic nerve, but no evidence of posterior scleritis. 7 months after presentation the patient was readmitted to hospital with a normocytic anaemia and renal impairment. A tap from the subretinal space in the left eye revealed malignant cells. Abdominal ultrasound scan showed widespread metastases as did the MRI brain scan, having both been normal at presentation. Adenocarcinoma was confirmed on liver biopsy. A primary site was never located and the patient died within a month of diagnosis.

**Conclusion** Scleritis is associated with many systemic conditions, but very rarely is it associated with metastic malignant disease as a presenting feature, as in this case. In addition, this case emphasizes the role of subretinal fluid examination and the importance and relevance of repeating investigations in an effort to establish a diagnosis.

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## Experimental study of the survival of metastatic cancer cells on organ cultured corneas: cell tracking and molecular biology

*DEB N (1), ABSI L (2), DUMOLLARD JM (3), CAMPOS-GUYOTAT L (1), GAIN P (1), THURET G (1)*
*(1) Laboratory " Cell survival and adhesion in cancers and grafts", EA3063 Faculty of Medicine, University Hospital Bellevue, Saint-Etienne*
*(2) Cornea Bank/ French Blood Centre Auvergne-Loir, Saint-Etienne*
*(3) Department of Pathology, University Hospital Bellevue, Saint-Etienne*

**Purpose** The transmission of cancer via corneal graft has been a subject of debate. To investigate its possible cellular mechanisms we studied the survival and adherence of 4 metastatic cancer cell lines in corneal organ culture (OC). We previously demonstrated their survival on donor corneas by in-vitro cell viability assay, histopathology and immunohistochemistry. Cell tracking and molecular biological studies were conducted for confirmation of these findings

**Methods** Varying inoculums (1000-1M) of cutaneous melanoma, epidermoid lung carcinoma, breast and colon adenocarcinoma cell lines, labelled with the fluorescent marker PKH 67 were seeded on the endothelial side of scientific human corneas, incubated for 6 hours to allow adherence, then stored in OC for 2 weeks. Cell adherence was then demonstrated by tracking with fluorescent microscopy. The Descemet Membrane (DM) was dissected and HLA typing done to search for cancer cell alleles. The DM was subjected to primary culture to confirm the proliferative capacity of adhered tumor cells.

**Results** Cell tracking revealed persistent cancer cell adherence on endothelium, further confirmed by presence of cancer cell HLA alleles. Primary culture of the DM and HLA matching showed proliferation of cells identical to the original tumour cell line

**Conclusion** Under laboratory conditions, tumor cells adhere to the corneal endothelium and stay viable in OC. Whether this happens in vivo on intact endothelium remains to be investigated. Considering that donor tissue from deaths due to malignancy constitute a significant percentage of eye bank reserves, further studies are required before commenting on the safety of such harvests.

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## Palpebral basal cell carcinoma: relation between 34-betaE12, bcl-2 and Ki-67 expression

*MISIUK HOJLO M (1), WOZNIAK ZM (2), BARANCEWICZ-LOSEK M (3), SZYBEJKO-MACHAJ G (3), MAJ J (3), BIENIEK A (3)*
*(1) Ophtalmology Wroclaw Medical University, Wroclaw*
*(2) Pathology, Wroclaw*
*(3) Dermathology, Wroclaw*

**Purpose** Basal cell carcinoma (BCC) is the by far most frequent neoplasm of palpebral tissues. The antibody 34-betaE12 stains selectively the keratins on the basal cells. Bcl-2 oncogene is an indicator of cell cycling with reduced apoptosis. Ki-67 is an antigen that corresponds to a nuclear protein expressed in phases G1, S, G2M. The aim of this study is to investigate the staining pattern of 34-betaE12, expression of cell proliferation markers (bcl-2, Ki-67) and influence of environmental factors to develop palpebral BCCs.

**Methods** 34-betaE12, bcl-2 and Ki-67 was studied immunohistochemically in 64 cases of BCCs (30 non-aggressive-BCC1, 34 aggressive-BCC2). The staining of 34-betaE12 were analysed and called 'focal' and 'diffuse.' Mean of proliferation index and distribution pattern (Ki-67,bcl-2) were assessed. The staining pattern of 34-betaE12, bcl-2 and Ki-67 expression were compared with architectural pattern, neoplastic progression and environmental factors.

**Results** All the BCC1 showed distinct cytoplasmic staining for bcl-2. The intensity of staining ranged from intermediate to high, with only four cases showing low positivity. Among BCC2, seven of the 34 cases showed positivity for bcl-2. Ki-67 index and Bcl-2 expression was directly correlated with the BCC1 and favourable clinical follow-up ($p<0.01$). Distribution and expression of Ki-67 paralleled with to the staining pattern of 34-betaE12. Influence of ultraviolet radiation exposure alone and in connection with smoking, immunosuppression, occupational factors, arsenic and ionizing radiation exposure, was described.

**Conclusion** 34-betaE12, bcl-2 and Ki-67 are useful indicators in diagnosis and progression of palpebral BCC.

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## Use of Mitomycin C (MMC) in the treatment of primary acquired melanosis of the conjunctiva

*MISSOTTEN GS, DE KEIZER RJW, DE WOLFF-ROUENDAAL D*
*Ophthalmology, Leiden*

**Purpose** Evaluation of the use of MMC for primary acquired melanosis with atypia (PAM+).

**Methods** Local outcome, complications and follow-up of 10 patients, treated with MMC 0.02% or 0.04% for PAM+ were evaluated. MMC was applicated for four weeks, four times daily.

**Results** After two cycli of MMC, melanosis disappeared in all patients. In three patients a progression to melanoma occured within the follow-up time of 40 months, which ended in an exenteration in one case. All patients developed keratoconjunctivitis during treatment, disappearing wihtin 2 weeks after treatment. In one patient a slight symblepharon developed after 2 cycli of MMC.

**Conclusion** Mitomycin C has a value in the management of diffuse and long-standing melanosis with atypia, but can not prevent recurrences, nor the progression to melanoma. The treatment is well-tolerated with our treatment protocol.

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## Effects of the Raf inhibitor BAY43-9006 in human uveal melanoma cell

*MOURIAUX F (1, 2), CALIPEL A (2), GLOTIN AL (2), MASCARELLI F (2)*
*(1) CHU service d'Ophtalmologie, Caen*
*(2) INSERM U598, Paris*

**Purpose** Acquisition of autonomous cell growth involves autocrine stimulation of growth factors-induced ERK1/2 activation, suggesting that wild-type Raf isoforms may be involved in uveal melanoma growth. We investigated the potential therapeutic role of the Raf inhibitor BAY43-9006 in controlling uveal melanoma cell proliferation.

**Methods** Four melanoma cell lines were used in this study. Cells were treated with various concentrations of BAY43-9006 and stimulated with serum. Cell proliferation was analyzed by the MTT method and activation of the ERK1/2 signalling pathway was determined by western blotting using specific antibodies against activated ERK1/2 and non-activated ERK1/2.

**Results** BAY43-9006 reduced cell proliferation in a concentration-dependent manner. Surprisingly, the efficiency of BAY43-9006, quantified by determining the concentration of BAY43-9006 necessary to inhibit cell proliferation by 50% (IC50), did not depended on the expression of the V599EB-RAF phenotype. We then investigated the signalling pathway of BAY43-9006 in uveal melanoma. BAY43-9006 inhibited uveal melanoma proliferation via blocking the ERK1/2 signalling pathway

**Conclusion** We provide the first evidence that BAY43-9006 inhibited uveal melanoma cells proliferation by the ERK1/2 signalling pathway. These study strongly suggests that this new pharmacological component could be used to treat uveal melanoma.

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## Individual calibration of sources in episcleral brachytherapy

*DE FRUTO BARAJA JM (1), SAORNIL MA (2), ALMARAZ A (3), LOPEZ-LARA F (4), DEL CASTILLO A (4), GIRALDO A (5)*
*(1) Radiotherapy (Medical Physics), Hospital Clínico Universitario, Valladolid*
*(2) Ocular Oncology, Hospital Clínico Universitario, Valladolid*
*(3) Epidemiology and Preventive Medicine, Hospital Clínico Universitario, Valladolid*
*(4) Radiotherapy, Hospital Clínico Universitario, Valladolid*
*(5) Retina, Hospital Clínico Universitario, Valladolid*

**Purpose** To assess the differences between individual or average calibration of Iodine 125 sources in episcleral brachytherapy. To evaluate the influence in the dose distribution and secondary effects.

**Methods** Three real choroidal melanoma cases are analized with different location ( nterior to,at and posterior to the equator) Dosimetry was performed with the Bebig brachytherapy planning system and the applicators employed were Ropes of sizes 11, 15 and 18 mm with 5, 10 and 14 sources of I-125. To assess the differences, ratio of average and individual calibration at sclera, optic disk, lens and macula was calculated.

**Results** The ratio of average and individual calibration for each case was as follows: Case 1 (Tumor anterior to the equator): sclera 0,999; optic disk 1; lens 0,998; macula 1,001.Case 2 (Tumor at equator): sclera 0,997; optic disk 0,997; lens 1,003; macula 0,998. Case 3 (Tumor posterior to the equator): sclera 0,999; optic disk 1,009; lens 1.003; macula 1,007.

**Conclusion** The ratio between average and individual calibration is negligible for any location studied. Indivividual strenght calibration, is more complicated and time consuming and may leads to increase risk of mistakes. However individual calibration of the sources remain necessary for quality assurance procedures.Partially funded by grant "José Mª Aguilar Bartolomé" and FIS 01/1664 (Ministerio de Sanidad y Consumo, España)

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## Transpupillary thermotherapy in choroidal melanoma with vitreous haemorrhage as its first clinical sign: case report

*VIDE ESCADA EICKMANN A, NASCIMENTO J, CASTANHEIRA-DINIS A*
*Instituto de Oftalmologia Dr. Gama Pinto, Lisboa*

**Purpose** A case report of choroidal melanoma and hemovitreous which showed remission after vitrectomy via pars plana (vvpp) and transpupillary thermotherapy with diode laser 810 nm (TTT).

**Methods** The subject, female, 72 years old, with no relevant past medical history, presented with sudden visual acuity loss (VA) in the right eye (RE). VARE was less than 1/20 and a hemovitreous RE was diagnosed. The RE ultrasound showed the presence of a homogeneous, fusiform, justapapillar inferior nasal mass, suggestive of choroidal melanoma. General analytic and imagiologic screenings were negative for primary tumour and metastasis. A vvpp was performed with visualization of the mass, associated with a tear of the overlying retina. Peri-tumoral endolaser and fluid collection for cytopathological exam were performed. Three weeks later facoemulsification with intraocular lens implantation was performed and the tumor dimensions calculated by ultrasound (6.09 x 6.75 x 2.72 mm3). Two sessions of over-dosed TTT were made at 24 and 48 hours post-op. The patient was reviewed at 24, 48 and 72 hours, week 1 and months 1, 2, 3, 4, 5, 6, 9, 12, 15 and 18 post-op.

**Results** After treatment, total macroscopic regression of the lesion was observed, with non-measurable residual scar tissue. VARE= 1/20. TTT was complicated by inferior branch occlusion, treated with laser 6 months after. The subject remains in remission until the present day (at 20 months follow-up).

**Conclusion** TTT as a single therapy can be used in the treatment of small choroidal melanoma lesions, being both an economical and non-aggressive therapy.

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## Detection of uveal melanoma cells in peripheral blood using the melan-A antigen

*MORILLA A (1), OREA M (1), BLANCO G (1), SAORNIL MA (1), ORDOñEZ JL (1), MENDEZ MC (1), BURNIER JR MN (2)*
*(1) IOBA-University of Valladolid, Valladolid*
*(2) The Henry Witelson Ophthalmic Pathology Lab. McGill University, Montreal*

**Purpose** To develop a highly sensitive and specific test, for the early detection of circulating melanoma cells in peripheral blood. The objectives of this study are: 1.-To determine MART1/MelanA mRNA with PCR technique, in different dilutions of SP6.5 human uveal melanoma cell line in culture medium and blood . 2.-To detect MART 1/ Melan A mRNA in peripheral blood, in a uveal melanoma model in the rabbit.

**Methods** In vitro: conventional cell culture methodology was used for the human uveal melanoma cell line SP6.5. Serial dilutions of tumor cells (106 to 1cell) in cultured medium and blood were used to determine MART1/Melan A mRNA using RT-PCR techniques and gene products were amplified by a nested PCR with specific primers. In vivo: 106 cells of uveal melanoma cell line (SP6.5) were implanted in the suprachoroidal space of 20 male albino rabbit eyes, which were immunosuppressed with daily injections of Cyclosporin A during 11 weeks. Ophthalmoscopy evaluation, weighting of animals to CsA dosage, and extraction of 2mL of blood (RT-PCR technique was used for detecting MART1/Melan A) were done weekly.

**Results** In vitro: The limit of detection of melanoma cells was 1 cell in cultured medium and 3 cells/mL in human blood. In vivo: 1.-We are able to detect tumor cells in peripheral blood of rabbits. 2.-animal model: 55% of the animals died during the first week, so they were not included in the study. 100% of the rabbits developed primary tumor and 5 of 9 (56%) with micrometastases.

**Conclusion** A sensitive level of detection of melanoma cells in cultured medium and blood has been achieved. Uveal melanoma cells can be detected in peripheral blood in this animal model.

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## Visual function and subjective perception of visual ability following vitrectomy and epiretinal membrane peel surgery

*GHAZI-NOURI SM (1), TRANOS PG (1), RUBIN GS (2), ADAMS ZC (1), CHARTERIS DG (1)*
*(1) Moorfields Eye Hospital, London*
*(2) Institute of Ophthalmology, London*

**Purpose** To study the functional and anatomical outcome of epiretinal membrane (ERM) peel and to evaluate its effect on patients' Health-Related Quality of Life (HR-QOL) and to explore the association between self reported HR-QOL and traditional measures of visual function.

**Methods** The National Eye Institute 25-Item Visual Function Questionnaire (VFQ-25) and the 36-Item Short-Form Health Survey (SF-36) were self-administered by 20 patients before and 4 months after ERM peel. Near and distant LogMAR visual acuity (VA), contrast sensitivity and metamorphopsia were recorded pre and post-operatively. Multi-item scales rating different aspects of HR-QOL were compared pre and post surgery and their correlation to traditional methods of visual outcome evaluation was analysed.

**Results** Improvement in mean LogMAR VA by a mean of 3 ± 13 letters and 4 ± 18 letters for distance and near respectively failed to reach statistically significant levels ($p>0.05$). However, 8 (40%) subjects' VA improved by 2 or more ETDRS lines and 9 eyes (45%) reached a final VA of 6/18 or better. Metamorphopsis decreased significantly ($p=0.019$) with 4 patients reporting no distortion at 4 months. There was significant improvement in VFQ-25 mean scores for the general vision ($p=0.025$), distance activities ($p=0.05$) and composite score ($p=0.03$). Baseline binocular visual acuity was significantly correlated with baseline VFQ-25 composite score ($r=0.631$, $p=0.004$).

**Conclusion** In this case-series, ERM surgery appears to have a beneficial effect on patients' subjective perception of visual function as shown by higher composite scores in VFQ-25 and improved metamorphopsia in the absence of significant improvements in mean LogMAR VA.

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## Sub-Tenon's anaesthesia for vitreoretinal surgery in patients receiving oral anticoagulation

*ELLIES P (1), BEJJANI RA (2), ELLIES E (3)*
*(1) Centre d'ophtalmologie Bld de Strasbourg, Toulouse*
*(2) Ophthalmology, Hotel-Dieu, Paris*
*(3) Service d'anesthesie, Hopital Purpan, Toulouse*

**Purpose** To assess the safety and efficacy of sub-Tenon's anaesthesia in patients undergoing retinal surgery while receiving oral anticoagulation

**Methods** 18 eyes of 18 patients undergoing retinal surgery underwent Sub-Tenon's anaesthesia while receiving oral anticoagulation (Anti-vitamin K). The International Normalised Ratio ranged from 2 to 3 for all patients. Injections were performed with a curved 25-mm sub-Tenon anaesthesia cannula. Patients received 3 drops of topical tetracaine 2 minutes prior to the sub-Tenon injection, 0.5 mg/kg of propofol IV. Ocular akinesia and analgesia scoring system were standardised, as were supplementary injection protocols. Patients were followed up daily for 3 days after surgery, then weekly for 6 weeks.

**Results** Rhegmatogenous retinal detachment (RD) repair (11 eyes of 11 patients) and epiretinal membrane (EM) removal (8 eyes of 8 patients) were performed. 7 RD patients were treated with scleral buckling and cryopexy, and 4 patients were treated with vitrectomy, endolaser photocoagulation, and complete fluid-gas exchange. No external drainage of subretinal fluid was performed.The EM patients underwent pars plana vitrectomy and peeling of the membrane. Sub-Tenon block was effective for all patients in terms of akinesia and analgesia.No major peroperative or postoperative complications related to abnormal bleeding were observed. A moderate choroidal detachment after episcleral buckling was observed in 3 eyes of 3 patients.

**Conclusion** Sub-Tenon's anaesthesia is a safe and effective technique for vitreoretinal surgery in patients receiving oral anticoagulation; therefore it may be not mandatory to discontinue oral anticoagulation prior to vitreoretinal surgical procedures in these patients.

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## Intraretinal proliferative vitreoretinopathy. Immunohistochemical study of peripheral retinectomies

*PASTOR JC (1), DE LA FUENTE MA (1), MENDEZ MC (1), SANABRIA RM (2), GARCÍA-ARUMÍ J (3)*
*(1) Instituto Universitario de Oftalmobiología Aplicada (IOBA), Valladolid*
*(2) Servicio de Oftalmología. Hospital Universitario Pio Del Rio Hortega, Valladolid*
*(3) Servicio de Oftalmología. Hospital Vall de Hebrón, Barcelona*

**Purpose** To identify the nature of the intraretinal process and cells involved in the intraretinal proliferative vitreoretinopathy (iPVR).

**Methods** We have studied 13 retinectomy specimens from patients with retinal detachment and iPVR. 1 normal retina from the eye bank and 4 enucleated eyes with end stage PVR were used as controls. The immunohistochemical study was made with a streptavidine-biotin amplified technique conjugated with alkaline fosfatase using fastred as chromogen. We tested antibodies against: vimentin, citokeratins, glial fibrillary acidic protein (GFAP) and CD68.

**Results** Intraretinal gliosis was observed in 12 out of 13 samples (92.3%). All 13 retinectomy specimens (100%) were anti-GFAP positive. 10/13 cases (76.9 %) had remnants of ERM all of them anti-GFAP negative. 3/10 ERMs (30%) were anti-CKs positive. CD68 positive cells were found on 11/13 (84.6%) of the specimens.

**Conclusion** The intraretinal shortening observed in this type of PVR is due at least in part to Müller cells gliosis, a different mechanism of what is present in the periretinal proliferation of other types of PVR. We have also detected macrophage-like cells that could play an important role on the iPVR pathogenesis.

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## Intraocular immunopathology following silicone oil tamponade

*WICKHAM LJ (1), ASARIA RHY (1), LUTHERT PJ (2), CHARTERIS DG (1)*
*(1) Moorfields Eye Hospital, London*
*(2) Institute of Ophthalmology, London*

**Purpose** Analyse the intraocular distribution of silicone oil and associated inflammatory reaction.

**Methods** Immunohistochemical analysis with a panel of monoclonal and polyclonal antibodies, of 38 epiretinal membranes, 15 proliferative vitreoretinopathy membranes, 35 retro-oil membranes, 19 retinectomies, 15 retro-oil retinectomies and 18 globes.

**Results** Emulsified silicone oil, frequently within macrophages, was present in the drainage angle and in membranes on the ciliary body, iris and retina. Macrophages were significantly increased in retro-oil epiretinal membranes ($p<0.001$). Lymphocytes were infrequent.

**Conclusion** Silicone oil and associated macrophages are present in intraocular membranes but not within the retina.

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## Perfluorocarbone liquid as a temporary tamponade for complicated inferior retinal detachments

*KUFFOVA L, BIESTER S, FORRESTER JV*
*Dept of Ophthalmology, IMS, University of Aberdeen, Aberdeen*

**Purpose** To assess the efficacy of the temporary internal tamponade with perflourocarbone liquid for complicated cases of inferior retinal tears with retinal detachment.

**Methods** Patients with complicated inferior retinal detachment due to the presence of proliferative vitreoretinopathy (PVR) underwent vitreoretinal surgery using perflourocarbone liquid (PFL) as temporary endotamponade.

**Results** In a period of 18 months 21 patients (10female/11male; aged 26-79, mean 53) underwent pars plana vitrectomy with retinopexy and PFL endotamponade as a primary procedure (11 patients, 53%) or as a repeated procedure (10 patients, 47%). PFL was retained in the operated eye for 6-28 days (mean 19,8 days). During this period elevated intraocular pressure was found in 18 patients (85%), which required topical anti-glaucoma therapy. Ten patients (47%) also required systemic anti-glaucoma therapy. To control intraocular inflammation systemic steroids were used in 16 patients (76%). After removal of the PFL 10 patients (47%) did not require further endotamponade. However, in 11 patients (53%) silicone oil 1000 centistokes was used as a long-term endotamponade. The retinal status 6-9 months after surgery was stably re-attached in 16 patients (76%).

**Conclusion** This study showed benefit of temporary use of PFL in the attempt to stabilize retina in the presence of inferior retinal tears with PVR. Use of PFL is associated with increased IOP and intraocular inflammation. A postoperative protocol including intensive topical steroid, atropine and anti-glaucoma therapy with systemic steroids offers the best opportunity to steer the patients through the initial 3-4 weeks post surgery.

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## The relevance of the adhesive properties of leukocytes in the Diabetic Retinopathy

*SERRA AM (1), WADDELL JM (1), COTTER MA (2), DUNCAN L (1), FORRESTER JV (1)*
*(1) Ophthalmology, Aberdeen*
*(2) Biomedical Sciences, Aberdeen*

**Purpose** Several studies postulate that inflammation might play an important role in the development of Diabetic Retinopathy. It has been reported that leukocytes adherent to the retinal endothelial cells and adhesion molecules are up regulated in animal models of induced diabetes. The aim of this work was to investigate the expression of adhesion molecules and the adhesion of leukocytes in diabetic mice.'

**Methods** Diabetes was induced in mice by intraperitoneal injection of streptozotocin. Immunocytchemistry was performed on whole flat mounted retinae and on spleen section of normal and diabetic mice. Leukocytes obtained from spleen and bone marrow of normal and diabetic mice were analysed for expression of activation markers, adhesion molecules and chemokines by flow cytometry, western blot and PCR. ELISA technique was used to quantify adhesion molecule ligands in the blood samples of these mice.

**Results** Leukocyte adhesion to retinal vessels was increased in diabetic mice. The expression of various parameters was increased in leukocytes from diabetic mice compared to normal.

**Conclusion** These results support the concept of inflammatory mechanisms underlying the pathogenesis of some aspects of Diabetic Retinopathy.Support by Development Trust of the University of Aberdeen and Astra Zeneca.

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## Inducible nitric oxide synthase is involved in increased leukocyte adhesion to retinal vessels induced by diabetes

*LEAL EC (1, 2, 3), MANIVANNAN A (4), COTTER M (5), AMBROSIO AF (1, 6), FORRESTER JV (3)*
*(1) Center of Ophthalmology of Coimbra, IBILI, Faculty of Medicine, University of Coimbra, Coimbra*
*(2) PDBEB, Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra*
*(3) Ophthalmology, University of Aberdeen, Aberdeen*
*(4) Biomedical Physics & Bioengineering, University of Aberdeen, Aberdeen*
*(5) Biomedical Sciences, University of Aberdeen, Aberdeen*
*(6) Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra*

**Purpose** There is accumulating evidence to indicate that inflammation might play an important role in the development of diabetic retinopathy (DR). Previous work has shown that inducible nitric oxide synthase (iNOS) levels and nitric oxide levels are increased in diabetic retinas. In this work, we aimed to investigate the role of iNOS in leukocyte adhesion to retinal vessels in diabetes.

**Methods** Diabetes was induced in mice by intraperitoneal injection of streptozotocin. Leukocytes obtained from spleens of normal mice were labeled with calcein-AM and transferred to normal, diabetic, iNOS knockout (iNOS KO) or diabetic iNOS KO mice. After Evans Blue infusion, flat mounted retinas were examined for leukocyte adhesion by confocal microscopy. In vivo leukocyte adhesion was assessed using real time scanning laser ophthalmoscopy and images were recorded on DVD-R for leukocyte adhesion analysis.

**Results** Leukocyte adhesion to retinal vessels was increased in diabetic mice. In diabetic iNOS KO mice leukocyte adhesion was decreased compared with diabetic mice. There was no difference in leukocyte adhesion in normal mice compared with iNOS KO mice.

**Conclusion** These results clearly demonstrate that iNOS contribute to increased leukocyte adhesion to retinal vessels caused by diabetes.Supported by: FCT, Portugal (POCTI/CBO/38545/2001; SFRH/BD/9686/2002) and FEDER.

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## Intravitreal triamcinolone acetonide as an adjuvant therapy to panretinal photocoagulation for proliferative retinopathy with high risk characteristics in type 1 diabetes: case report with 1 year and 4 months follow-up

*KYTÖ JP (1), KROOTILA K (1), LUMISTE E (1), HIETALA K (1), PALOHEIMO M (2), SUMMANEN PA (1)*
*(1) Department of Ophthalmology, University of Helsinki, Helsinki University Central Hospital, Helsinki*
*(2) Department of Anaesthesiology, University of Helsinki, Helsinki University Central Hospital, Helsinki*

**Purpose** To describe a treatment protocol to deliver panretinal photocoagulation that may avoid further deterioration of vision in patients with type 1 diabetes mellitus with proliferative retinopathy with high risk characteristics for severe visual loss and cystoid macular edema.

**Methods** Fundus photography, measurement of foveal thickness with optical coherence tomography and best corrected visual acuity (BCVA) determined by ETDRS chart were measured before and after treatment in a 28-year-old man.

**Results** Over 9 weeks, BCVA improved from 0.05 to 0.25 and the number of letters read at 2 m from 4 to 39 after panretinal photocoagulation and adjuvant intravitreal 4.8 mg triamcinolone injection under intraconal anaesthesia. Foveal thickness decreased from 691 to 239 µm and cysts disappeared by 15 weeks. By 22 weeks, foveal thickness increased to 282 µm and small cysts reappeared, and the number of letters read fell to 30 and eventually 21. The treatment was repeated at 7 months and CME decreased from 625 to 249 µm. Intraocular pressure increased and required medication. Cataract developed and was operated at 1 year and 4 months. BCVA improved to 0.25 and the number of letters read at 36. This eye continued to be the better eye. BCVA of the fellow eye was CF at 0.5 m.

**Conclusion** Panretinal photocoagulation and adjuvant intravitreal triamcinolone injection under intraconal anaesthesia represents a feasible treatment option in cases when pain during laser treatment and impairment of vision afterwards due to CME result in poor compliance with standard laser treatment under topical anaesthesia.

## ▪ 409
## Foveal neovascularization in diabetic retinopathy

*SUMMANEN PA, TOMMILA P, LUMISTE E*
*Dept. of Ophthalmology, University of Helsinki, Helsinki*

**Purpose** To describe foveal neovascularizations (fovNV) in diabetic patients and to report their outcome and effect on visual acuity (VA).

**Methods** Among diabetic patients examined and treated for diabetic maculopathy and/or retinopathy (rp) in diabetic outpatient clinic, patients with suspicion of new vessels along the foveal avascular zone were included in this clinical series. Fluorescein angiography (FA) and optical coherence tomography (OCT) were performed to confirm the diagnosis.

**Results** Eight patients (M/F=5/3), all but one with type 1 diabetes, with the mean duration of DM of 21 yrs and the mean age of 30 yrs, were diagnosed to have fovNV. In OCT fovNVs appeared as tufts and in FA rose from perifoveal capillaries of enlarged foveal avascular zone, filled with fluorescein and leaked. FovNV was unilateral in all but one patient. The index eye had been treated for proliferative rp in 6, for severe nonproliferative rp in 2 eyes, and in one eye fovNV was found prior to laser treatment. The average number of scatter burns was 5500. VA was >0,7 in 4 and 0,3-0,6 in 5 eyes. After the median follow-up of 1 yr (range 0,5-2,5 yrs) fovNV has increased in one eye. No macular edema or haemorrhages have occurred and the latest VA was >0,7 in 6 and 0,4-0,6 in 3 eyes. At the time of diagnosis, HbA1c was on average 9,5% (range 8-11) and four patients had medication for hypertension.

**Conclusion** Retinal new vessels occur at the optic disc and/or near the major nasal and temporal vascular arcades. The fovea seems to have resistance for their growth even if marked foveal capillary closure occurs. The most probable cause of this unusual new vessel growth in 8 patients is poor glycemic control. No significant growth has occurred in fovNVs and even if they leak in FA, they have not impaired vision.

## ▪ 410
## Antiphospholipid antibodies and retinal thrombosis

*MANGANELLI C, TURCO S, BALESTRAZZI E*
*Dept. Ophthalmology - Catholic University of Sacred Heart, Roma*

**Purpose** To describe the presence of antiphospholipid antibodies and other immunologic abnormalities in patients with occlusive retinal vascular events without conventional risk factors.

**Methods** Eight patients (5 F and 3 M, mean age 42 years, range 26-52 years) affected by retinal vascular occlusion without usual risk factors were evaluated for antiphospholipid antibodies (anticardiolipin and lupus anticoagulant antibodies) and other immunological abnormalities.

**Results** All patients showed positivity for antiphospholipid antibodies (anticardiolipin antibodies in 2 cases and lupus anticoagulant antibodies in 6 cases). Other immunological abnormalities were also found (antinuclear antibodies, elevation of circulating immune complexes, complement deficiency, positive rheumatoid factor, positive C-reactive protein). In 5 case more than 3 parameters were altered.

**Conclusion** The presence of anticardiolipin antibodies in patients with vaso-occlusive retinopathy without conventional risk factors has important diagnostic and therapeutic implications. A systematic research for specific antiphospholipid antibodies in retinal thrombosis can be recommended.

## ▪ 411
## Isolating triamcinolone acetonide particles for intravitreal use with different pore size filters

*RIGAL SASTOURNE J-C (1), MULLOT H (2), PAYEN C (3), GENTES P (3), BORNE-PONS M (2), RENARD JP (1), MAURIN JF (1)*
*(1) Ophthalmology, Hopital du Val de Grace, Paris*
*(2) Pharmacy, Hopital Begin, Saint-Mandé*
*(3) Pharmacy, Hopital du Val de Grace, Paris*

**Purpose** Triamcinolone acetonide intravitreal injection of 20 mg/0.1 ml has become increasingly popular for the treatment of cystoid macular oedema in ocular desorders. Two standard preparations have been described: after sedimentation or after microfiltration and excipient removal. The aim of this study was to develop a third method based on biggest particles isolating and to test its pharmaceutical performances.

**Methods** The content of a commercially available Triamcinolone acetonide vial (KENACORT 80 mg/2 ml) was homogenised, loaded into a syringe and passed through a filter. Different pore size filters (respectively 20, 30 and 40 µm) have been tested in order to isolate the biggest particles with an amount of triamcinolone acetonide closed to 20 mg. Dosage of triamcinolone were assessed quantitatively by weighing crystals after incubator drying.

**Results** Recovery results obtained with different pore size filters are discussed. Pore size filters giving the best recovery results (closeness to 20 mg) were used to test reproducibility and predictability of the new preparation method.

**Conclusion** The two standard preparations commonly used are incompatible with good pharmaceuticals practices and the need for development of a more adapted preparation has been underlined by several authors. If clinical results obtained by injection of biggest crystals were promising, the user friendly method presented here could become a new standard.

## ▪ 412
## Wild-type and Sorsby's fundus dystrophy mutant TIMP-3 exhibit differential thermal stability

*MAJID MA (1), SMITH VA (1), BAKER AH (2), NEWBY AC (2), DICK AD (1)*
*(1) Ophthalmology, Bristol Eye Hospital, Bristol*
*(2) Cardiology, Bristol Royal Infirmary, Bristol*

**Purpose** Sorsby's fundus dystrophy (SFD) and Age Related Macular Degeneration (ARMD) are retinal diseases characterised by the respective accumulation of mutant and wild-type TIMP-3 in Bruch's membrane. Since the accumulation of TIMP-3 is an important factor in the pathological progression of these diseases the purpose of this study was to compare the thermal stability of wild type and SFD mutant TIMP-3.

**Methods** COS-7 cells were transduced with wild-type and SFD mutant TIMP-3 and the proteins harvested. Aliquots of these proteins were placed in PCR tubes and heated at 100oC for varying times in a thermal cycler. Residual TIMP-3 activity in heated samples was assessed by reverse zymography.

**Results** Reverse zymograms of wild type and SFD TIMP-3 proteins heated for 24 hours showed little TIMP-3 activity. Those of heated wild type TIMP-3 showed reduced activity after 3 hours at 100oC, especially of the 50 kDa band. SFD TIMP-3 proteins showed little change after heating for up to 7 hours.

**Conclusion** Wild type and SFD mutant TIMP-3 proteins show differential thermal stability. The increased stability of the mutant proteins is likely to contribute to the early and increased accumulation of TIMP-3 seen in SFD compared to ARMD eyes. This could explain the aggressive nature of the pathological changes seen in patients with SFD.

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## Retinal degeneration is delayed by eye pigmentation and heterozygosis in the P23H rat

*CUENCA N (1), PINILLA I (2), SALIVE Y (3), MARTINEZ-NAVARRETE G (1), LUND RD (3)*
*(1) Biotechnology, Alicante*
*(2) Miguel Servet Hospital, Zaragoza*
*(3) Ophthalmology, Utah*

**Purpose** P23H is a mutation in autosomal dominant retinitis pigmentosa.The purpose of this study is to compare the influence of pigmentation and homozygosis vs. heterozygosis on the retinal degeneration rate in the P23H rat because is an important factor to assess the effect of therapeutic approaches

**Methods** Cryostat vertical retinal sections of 30 albino homozygous P23H and pigmented heterozygous P23H rats were compared immunocytochemically at different time points. Specific antibodies were used to study the different retinal neuronal types and their connectivity. Images were obtained by confocal microscopy.

**Results** Photoreceptor layers were rapidly lost in the albino rat; which at P40 displayed only 3 photoreceptor layers. Pigmented rats show this same number of layers around P180. Bipolar and horizontal cell dendrites were clearly diminished at P21 in the albino rat, and at this stage rod bipolar axon terminals were already impaired. However, bipolar and horizontal cell dendrites in pigmented rats showed the first signs of degeneration around P60, whereas rod bipolar axon terminals maintained a well preserved morphology at all the studied time points. Double immunolabelling showed that the ratio between photoreceptors and bipolar cells conectivity was impaired at P21 in the albino rat; whereas in the pigmented rat this ratio diminished gradually but without showing a dramatic decrease at P180.

**Conclusion** Pigmentation and/or heterozygosis slow down retinal degeneration rate in P23H rats. The pigmented heterozygous P23H rat constitutes a good model for progressive and slow retinal degeneration, as it occurs in autosomal dominant retinitis pigmentaria.Support: NIH (EY14038), FIS (PI042399), ONCE, Fundaluce.

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## Cell-based therapy preserves photoreceptor connectivity in a model of retinal degeneration

*PINILLA I (1, 2), SALVÉ Y (3), CUENCA N (4, 2), LUND RD (2)*
*(1) Hospital Universitario Miguel Servet, Zaragoza*
*(2) Moran Eye Center, Salt Lake City*
*(3) Ophthalmology, Edmonton*
*(4) Biotecnology, Alicante*

**Purpose** To examine the effect of preventive subretinal cell injections in the RCS rat on the outer retina and synaptic connectivity between photoreceptors and their target neurons.

**Methods** Eight rats received subretinal injections of a human RPE cell line (ARPE19) at age P22; they were sacrificed at P90 and P120. Controls consisted of 6 unoperated rats sacrificed at the same time points and 3 non-dystrophic RCS rats at P30. All animals were immunosuppressed with oral cyclosporine. The OPL, ONL, INL and IPL were studied immunohistochemically and retinal function examined with ERG.

**Results** In areas preserved by injected cells, maintained organization was evident in all the studied layers. Photoreceptors were clearly preserved, with maintained cone morphology, expressing both opsins. Bipolar cells were well organized in the optimal rescue area with normal dendritic branching. Synapses were well preserved with respect to rod terminal/ bipolar pairing and clear cone synaptic sites. Synapses became less numerous away from the rescue area. Some sprouting of horizontal and bipolar dendrites could be seen in areas away the transplant, related to the loss of photoreceptors. ERG recordings showed at best the persistence of both a-and b-waves.

**Conclusion** Photoreceptor rescue following hRPE cell injections is accompanied by the maintenance of synaptic connectivity and preservation of ERG. hRPE transplantation in this animal model of retinal degeneration can delay anatomical and functional loss.Support: NIH (EY14038), FIS (PI042399), ONCE, Fundaluce.

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## Anatomical and functional differences between RCS rat and P23H retinal degeneration

*PINILLA I (1, 2), CUENCA N (3, 2), SALVÉ Y (4), LUND RD (2)*
*(1) Hospital Universitario Miguel Servet, Zaragoza*
*(2) Moran Eye Center, Salt Lake City*
*(3) Biotecnology, Alicante*
*(4) Ophthalmology, Edmonton*

**Purpose** To examine differences between two Retinitis Pigmentosa models, RCS rat and P23H rat, due to two different mechanisms (RPE and photoreceptor mutations respectively).

**Methods** 15 dystrophics RCS rats (rdy+p+) and 15 P23H Line 1 homozygote albino rats at different time points were studied immunohistochemically and by ERG recording. Results were compared with their respective controls: congenic non-dystrophic RCS rats (rdy-p+) and albino Sprague-Dawley (SD) rats.

**Results** Both animal models showed a similar degeneration rate. The main differences between the two models of degeneration were related to the appearance of the debris zone in the dystrophic RCS rats, which provided a substrate for the sprouting of bipolars and horizontal cells. This reached a peak at P90, but disappeared with age in a central to peripheral gradient. The P23H rat showed an early loss of dendrites in rod bipolars, with associated reduction of mGluR6 and rod-associated bassoon staining, but cone pathways seemed relatively unaffected. At P150 the remaining bipolar and horizontal cells dendrites kept interconnected in clusters associated with bassoon and mGluR6.While there was no sprouting as into the debris zone, ERG responses were clearly reduced in both types of degeneration already by P21.

**Conclusion** While initial loss of bipolar dendrites and markers of synaptic transmission are seen early in degeneration in both models, in RCS rats there is subsequent sprouting of bipolar and horizontal cells dendrites. Anatomical changes can condition therapeutic options as retinal transplantation.Support: NIH (EY14038), FIS (PI042399), ONCE, Fundaluce.

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## Intervention to reduce inappropriate fluorescein angiography requests

*RAJAK SN, CURRIE ADM, CASSWELL AG, ECKSTEIN MB*
*Sussex Eye Hospital, Brighton*

**Purpose** Fluorescein angiography (FFA) is performed in order to assist the diagnosis and treatment of a variety of ocular conditions. FFA is not without significant side effects and the decision to perform FFA should not be without good reason. We present a new tick-box FFA request form introduced after an audit performed to identify inappropriate FFA requests. We re-audited the requests to assess the effect of the form.

**Methods** The notes of 100 consecutive FFA patients were reviewed. The need for the FFA was compared with current evidence-based guidelines and discussed with two senior retinal consultants. A new request form, which demanded both a specific indication (e.g. age related macular degeneration) and relevant criteria (i.e. recent symptoms of distortion, visual acuity more than 6/60, less than half lesion obscured by blood) was developed and distributed to all requesters at The Eye Hospital. A further 100 consecutive FFA patients' case notes were reviewed for the appropriateness of the request.

**Results** The initial audit determined that 17% of FFA requests were inappropriate. The repeat audit after introduction of the new request form showed a reduction of inappropriate FFA requests. Requesters found the new form to be eductational in guiding requests and to be user-friendly.

**Conclusion** Prior to the introduction of the new FFA request form, one in five requests were unnecessary. The introduction of an evidence based fluorescein request form has reduced this number to a more acceptable level.

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## Transpupillary thermotherapy (TTT) in Retinal Angiomatous Proliferation (RAP)

*PELOSINI L, MCHUGH DA*
*King's College Hospital, Denmark Hill, London*

**Purpose** To report two cases of retinal angiomatous proliferation (RAP) treated successfully with subthreshold transpupillary thermotherapy (TTT).

**Methods** Case 1: A 71 year old female presented with a right visual acuity of 6/9 and RAP was diagnosed by dilated fundoscopy and fundus fluorescein angiogram (FFA). The lesion was treated with TTT (1´2mm´600mw´1min) Laser: Oculight SLx (810 nm infrared), Iridex, Ca USA. The patient reviewed at months 1, 3, 6, 13 and 19, and FFA imaging was repeated at 6 and 19 months post TTT.Case 2: An 83 year old male presented with left blurred vision and an acuity of 6/12. FFA and optical coherence tomography (OCT) showed a left RAP inferior to the fovea that was treated with TTT (2´0,5mm´300mw´1min). Follow up was carried out monthly for the first 3 months with repeated FFA and OCT imaging.

**Results** One month after treatment, case 1 showed signs of resolution of leakage and the vision was stabilised at 6/9. Follow up 19 months post TTT revealed new exudates that appeared to be consistent with recurrent RAP on FFA study. A second TTT was administered (1´2mm´600mw´1min), 3 months post TTT fundal examination showed a further reduction in leakage and the vision was stable at 6/9.Two months post TTT, case 2 showed decrease in macular leakage and thickness on FFA study and OCT analysis respectively. Visual acuity improved to 6/6.

**Conclusion** TTT was administered safely and stabilized the RAP lesions in the two cases discussed in this report. These cases provide further evidence that the mechanism of action of laser therapy for vascular lesions does not rely upon direct thrombosis, given the low absorption of infrared energy within haemoglobin.

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## Diffuse Laser for Drusen reduction in High risk age related maculopathy

*ADEWOYIN TA, SIVAGNANAVEL V, CHONG NHV*
*Laser and Retinal Research Unit,King's College Hospital University of London, London*

**Purpose** To determine if drusen reduction with diffuse diode laser using large spot size,low energy and long duration could be achieved safely in patients with age related macular degeneration who are at high risk of visual loss.

**Methods** Twenty-one patients were recruited in a prospective double masked randomized trial. Eligible patients had subfoveal choroidal neovascularisation (CNV) from AMD in one eye and significant drusen (>5 large drusen or >20 small drusen) in the fellow eye. Drusen were graded according to the modified International Classification of ARM. The control group received sham treatment with no energy applied. Colour fundus photographs were evaluated to determine reduction of drusen.

**Results** Analysis of the data at 12 months shows some reduction of soft drusen in the treatment group. No changes in hard drusen were noted. Visual acuities in the treated group showed a trend towards an improvement. There were no adverse symptoms reported in the treatment group.In the sham treatment group 2 patients suffered a drop in visual acuity,one developed CNV,the other developed a central retinal vein occlusion.

**Conclusion** Treatment of drusen with diffuse laser in patients at high risk of visual loss appears to be safe at 12months post treatment. Longer term follow up will determine whether this treatment strategy will be significant in the reduction of soft drusen and / or prevention of CNV formation.

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## Photodynamic therapy in combination with intravitreal triamcinolone for myopic CNV

*DEGENRING RF, JONAS JB*
*Dept of Ophthalmology, Fac. for Clinical Medicine Mannheim, Ruprecht-Karls-University, Heidelberg*

**Purpose** To report on the combined use of photodynamic therapy (PDT) plus intravitreal triamcinolone in myopic choroidal neovascularisation (CNV) refractory to PDT alone.

**Methods** A 35 individual years developed classic subfoveal CNV due pathologic myopia (-17.0 dpt) and visual acuity (VA) decreased from 0.7 to 0.3. He received 5 sessions of photodynamic therapy every 10 to 12 weeks, which was followed by reperfusion of the neovascular complex about 4 weeks after every treatment. Visual acuity dropped to 0.08 and increasing chorioretinal and retinal pigment epithelium atrophy was observed within the treatment area. At last he received an intravitreal injection of about 20 mg triamcinolone acetonide, followed by a 6th PDT 2 weeks later.

**Results** After combination therapy, during follow-up of 8 months no reccurrence was observed and no subretinal fluid was detected by angiography an optical coherence tomography. Visual acuity increased to 0.125 and metamorphopsia diappeared.

**Conclusion** Combination of PDT plus intravitreal triamcinolone may be beneficial in myopic CNV refractory to PDT alone, and may reduce number of retreatments necessary. Further studies are warranted.

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## Plasma complement C3adesArg is raised in neovascular age-related macular degeneration

*SIVAPRASAD S (1, 2), BAILEY TA (2), CHONG NHV (1)*
*(1) Kings College Hospital, London*
*(2) Cranfield BioMedical Centre, Bedfordshire*

**Purpose** Recent evidence suggests that the complement system may play a significant role in the pathogenesis of age-related macular degeneration (AMD). The central component of the complement system is C3. Activation of C3 results in its proteolytic cleavage into C3a and C3b. The anaphylatoxin C3a is rapidly converted to a more stable form, C3adesArg. Estimation of serum C3adesArg reflects complement activation, independent of individual complement component levels. This study estimated plasma complement C3adesArg in subjects with age related macular degeneration (AMD) compared to age-matched controls to determine the role of systemic complement activation in the pathogenesis of AMD.

**Methods** Plasma complement C3adesArg was determined using a competitive ELISA in three groups of subjects: a) 25 subjects with early age-related maculopathy (ARM) b) 25 subjects with neovascular (wet) AMD and c) control group of 15 subjects with no clinical evidence of age related changes at the macula.

**Results** There was no significant difference in plasma complement C3adesArg between controls and ARM. However, there was a trend of increased plasma complement C3adesArg in patients with neovascular AMD.

**Conclusion** This study shows that systemic activation of the complement system may contribute to the pathogenesis of neovascular AMD. Further work is required to establish the role of complement in AMD.

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## Effect of timolol maleate on subfoveal choroidal blood flow measured by laser Doppler flowmetry

*LINGUERRI S (1), PARMEGGIANI AL (1), FALSINI B (2), RIVA CE (1)*
*(1) Unità Operativa di Oftalmolgia, Università degli Studi di Bologna, Bologna*
*(2) Istituto di Oftalmologia, Università Cattolica S. Cuore, Roma*

**Purpose** To investigate the effect of timolol maleate on subfoveal choroidal blood flow in a group of normal subjects.

**Methods** In 9 healthy subjects with normal eye examination and average age of 38 (stdev = 17) years, a drop of either timolol maleate 0.5% or a placebo was administered in a masked fashion in one eye chosen at random. Two weeks later, the other drop was instilled in the same eye. Subfoveal relative velocity, volume and flow of blood (LDF parameters) in the choriocapillaries behind the fovea were measured for at least 1 min by laser Doppler flowmetry in both eyes at baseline and approximately every half hour during a 2-hour period following the instillation of the drop. Care was taken to obtain DC values of the Doppler signal as close as possible to each other between the recordings in the same eye. Brachial artery blood pressure, IOP and heart rate were determined at baseline and at the end of the measurements.

**Results** There was a drop of heart rate from an average of 80 to 70 bpm and a significant ($p = 0.006$) decrease in IOP by approximately 2 mm in the eye that received timolol. The percent changes in each LDF parameter during the 2 hours of measurement were not significantly different between the timolol and the placebo eye. For our normal subjects, the calculated smallest percent difference that the technique could detect between the LDF data obtained after timolol and after placebo was found to be approximately 5%.

**Conclusion** This study shows that the instillation of one drop of timolol 0.5% produces no detectable effect on subfoveal choroidal blood flow in normal subjects.

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## Combined photodynamic therapy and intravitreal triamcinolone acetonide for neovascular age-related macular degeneration associated with pigment epithelial detachment

*ELLIES P (1), BEJJANI RA (2)*
*(1) Centre d'ophtalmologie Bld de Strasbourg, Toulouse*
*(2) Ophthalmology, Paris*

**Purpose** To study the visual and angiographic outcomes of eyes treated with combined verteporfin photodynamic therapy (PDT) and intravitreal triamcinolone acetonide for neovascular age-related macular degeneration (AMD) associated with pigment epithelial detachment (PED).

**Methods** 7 eyes of 7 patients with AMD associated with choroidal neovascularization (CNV) and PED, and presenting a severe decrease of their BCVA (BCVA < 20/100) were treated with intravitreal injection of 4 mg of triamcinolone acetonide followed by PDT 2 to 3 months after. Initial and final BCVA were measured with a retroilluminated ETDRS chart. The need for retreatment was assessed based on fluorescein angiographic evidence of leakage at 3-month follow-up intervals. PED was documented by Optical Coherence Tomography (OCT).

**Results** Mean follow-up after PDT was 5.6 months +/- 2.3. No subretinal hemorrhage or retinal pigment epithelium tears were observed. No loss in visual acuity line were reported ; the mean change was a gain of 2 lines. No patient needed a retreatment. In all eyes PED subsided, partially (4 eyes) or totally in (3 eyes) at 3 months after IVT. An elevated intraocular pressure elevation (IOP) of >24 mmHg developed in 2 eyes during follow-up. No endophthalmitis was reported.

**Conclusion** The preliminary results in this limited number of patients document the efficacy in terms of BCVA improvement and PED subsidence of combined verteporfin PDT and intravitreal triamcinolone acetonide for neovascular AMD associated with PED.

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## Hsp90 inhibitor modulates ubiquitin–proteasome pathway linked protein aggregation in human ARPE–19 cells

*RYHÄNEN T (1), KAARNIRANTA K (1), KUUSISTO E (2), MIETTINEN R (2), SALMINEN A (2), TERÄSVIRTA M (1), UUSITALO H (1)*
*(1) Ophthalmology, Kuopio*
*(2) Neuroscience and Neurology, Kuopio*

**Purpose** Pathogenesis of age–related macular degeneration (AMD) involves to impaired degradation of membranous discs shed from photoreceptor outer segments and accumulation of lysosomal lipofuscin in RPE cells. In addition to lysosomal protein degradation, many cellular proteins are degraded in proteasomes. In response to various stresses, cells increase the expression of heat shock proteins (Hsps). They function as a molecular chaperones, in order to prevent the accumulation of cellular cytotoxic protein aggregates. Role of Hsp70, Hsp90 and proteasome inhibition were evaluated in cellular aggregation in human RPE cells (ARPE–19).

**Methods** Accumulation Hsp70 and ubiquitinated proteins were analyzed by Western blotting. Transmission electron microscopy was used to detect cellular organelles in ARPE–19 cells. Hsp70 and Hsp90 were localized by immunocytochemistry.

**Results** MG–132 proteasome inhibitor caused robust accumulation of Hsp70 protein and ubiquitinated protein conjugates in ARPE–19 cells. Electron microscopy analyses showed highly, in size and context, varied perinuclear protein aggregates in response to proteasome inhibition. Hsp70 but not Hsp90 colocalized with the protein aggragates. When the cells were subjected to Hsp90 inhibitor geldanamycin the amount of protein aggregates was clearly decreased.

**Conclusion** This study reveals that ubiquitin–proteasome pathway is an important way to control protein turnover in the RPE cells. In addition, Hsp70 and Hsp90 are closely related to regulation of the cytoplasmic protein aggregation.

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## The Effect of Posterior Juxtascleral Triamcinolone Acetonide on Choroidal Neovascular Growth After Photodynamic Therapy With Verteporfin For Age-Related Macular Degeneration

*VAN DE MOERE ASJ, TALKS SJ, SANDHU SS*
*Royal Victoria Infirmary, Eye department, Newcastle upon Tyne*

**Purpose** To assess if posterior juxtascleral application of 40mg triamcinolone acetonide(TAAC), given at the same time as initial photodynamic therapy(PDT) for predominantly classic choroidal neovascularization(CNV) related to age-related macular degeneration(AMD), affects lesion growth at three and six months.

**Methods** We compared two consecutive case series. The study group consisted of 38 eyes of 38 patients who received a posterior juxtascleral application of 40 mg TAAC together with their initial PDT treatment. The control group consisted of 73 eyes of 73 patients who were treated with PDT alone. All patients were reviewed at 1, 3 and 6 months. Outcome measures were change in total lesion size, area of leak, best corrected visual acuity, number of treatments and intraocular pressure.

**Results** There was significantly less growth of total lesion at 3 months (mean difference=2.47mm2, p=0.0002) and 6 months (mean difference=2.88 mm2, p=0.0134) in patients given TAAC with PDT compared to PDT alone. There was also a significantly smaller remnant area of leak at 3 months in the study group (mean difference=1.07 mm2, p=0.02). At 6 months, the remnant area of leak between the two groups became comparable (p=0.86). Mean number of letters lost on the LogMAR chart at 6 months was 9.1 ± 13.1 letters in the study group compared with 12.4 ± 16.3 letters in the control group (p=0.30). Intraocular pressure was raised in 4 of 38 eyes. Less re-treatments were required in the TAAC with PDT group, 2.03 compared with 2.47 (p=0.006).

**Conclusion** Conclusions: Posterior juxtascleral placement of TAAC with PDT at baseline significantly reduces CNV growth at 3 and 6 months.

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## Photodynamic therapy for subfoveal choroidal neovascularization. A coulor doppler study

*CELLINI M, PAZZAGLIA A, ZAMPARINI E, CAMPOS EC*
*Department of Surgery and Transplant "A. Valsalva", Bologna*

**Purpose** To perform a color Doppler analysis of the effects on ocular blood flow of the photodynamic therapy (PDT) for subfoveal choroidal neovascularization (CNV) in age related macular degeneration (AMD).

**Methods** Ten patients aged from 64 to 78 (mean 72.4 yrs) with subfoveal CNV due to AMD were assessed in a mask fashion by color Doppler imaging (CDI) within 24 hours before, 1 week after and 2 months after undergoing PDT. All subjects were treated with verteporfirin at a drug dose of 6 mg/m2 body surface area and a light dose of 50 J/cm2. Patients included in the study underwent a CDI examination of the ophthalmic artery (OA) and posterior ciliary arteries (PCA's) with an AU 580 Asynchronous-Hitachi analyser and a 7.5 MHz linear probe. A statistical analysis was made of the peak systolic velocity (PSV) and resistive index (RI) in the OA in the OA and PCA's using the Student "t" test for paired data.

**Results** In the PCA's there were no statiscally significant changes observed in the peak systolic velocity (PSV) and in resistive index (RI) at 1 week. After 2 months the PSV was 9.358+0.779 cm/sec vs 8.175+0.412 cm/sec ($p<0.003$) and the RI increase 0.687+0.028 vs 0.714+0.015 ($p<0.008$). There were no statistically significant differences in the OA blood flow indices at 1 week and 2 months after treatment.

**Conclusion** PDT is associated with decreased volumetric blood flow in the posterior ciliary arteries that supply the choroids two months after treatment. This study suggest that PDT could lead to alteration in choroidal blood flow and this appears to influence the visual outcome of the treatment.

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## Retinal Vessel Diameter in Asymmetric Age-related Macular Degeneration

*SANDHU R, SIVAPRASAD S, MADILL S, CHONG V*
*Retinal Research Unit, King's College Hospital, London*

**Purpose** Ocular perfusion abnormalities have been proposed in the pathogenesis of age related macular degeneration (AMD).Recent studies suggest that structural retinal arteriolar changes may contribute to age related maculopathy (ARM) progression. The aim of our study was to evaluate retinal vascular diameters in each of the fellow eyes of patients with asymmetric AMD.

**Methods** Patients with asymmetric AMD were divided into three groups. Group 1 (20) with choroidal neovascular membrane (CNV) in one eye and drusen in the fellow eye; Group 2 (15) with disciform scar in one eye and drusen in the fellow eye; Group 3 (7) with CNV in one eye and disciform in the other eye. Computer-assisted grading (similar to the Wisconsin system) was performed from a digital fundal image centered on the disc to determine the average diameter of retinal arterioles (central retinal arteriolar equivalent or CRAE) and venules (central retinal venular equivalent or CRVE) and their ratio (AVR) in fellow eyes of patients with asymmetrical AMD. The macular area was cropped to the masked observer. Student's t-test was used to compare the values in fellow eyes with asymmetric AMD.

**Results** Group 1: mean AVR was 0.37 (mean CRVE=217.15, mean CRAE=77.16) in eyes with CNV and 0.32 (mean CRVE=223.24, mean CRAE=68.86) in fellow eyes with drusen, p=0.16; Group 2: mean AVR was 0.27 (mean CRVE=223.64, mean CRAE=60.04) in eyes with disciform scar and 0.27 (mean CRVE=224.45, mean CRAE=60.35) in fellow eyes with drusen, p=0.9; Group 3: mean AVR was 0.29 (mean CRVE=220.01, mean CRAE=64.16) in eyes with CNV and 0.29 (mean CRVE=223.57, mean CRAE=64.78) in fellow eyes with disciform scar, p=0.96.

**Conclusion** Although the results are not statistically significant, there is a trend in Group 1 suggesting an increased AVR in eyes with CNV (0.37) as compared to fellow eyes with drusen (0.32). This increase in ratio appears to be due to an increase in arteriolar diameter in eyes with CNV.

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## Optical coherence tomography findings following combined PDT with verteporfin and intravitreal triamcinolone acetonide in exudative AMD

*TEUS MA (1, 2, 3), FIGUEROA M (1, 4), GÓMEZ MI (1), ORTEGA M (1)*
*(1) Vissum Hospital Ofaltalmológico de Madrid, Madrid*
*(2) Universidad de Alcalá, Alcalá de Henares*
*(3) Hospital Príncipe de Asturias, Alcalá de Henares*
*(4) Hospital Ramón y Cajal, Madrid*

**Purpose** To assess morphological changes in the macular area after Photodynamic Therapy (PDT) combined with intravitreal triamcinolone acetonide (iTAAC) using OCT3.

**Methods** Prospective, controlled, observer-masked study using standard PDT with (study group) or without (control group) 4 mg of iTAAC,. Results: 18 patients were included in the study group and 15 in control group. In both groups, macular parameters increased two days after treatment and then showed a decrease at two weeks. 1.5 months post-op the decrease in macular volume was higher in study group (p=0.04). Conclusion: Combined PDT and iTAAC modifies the macular thickness changes induced by standard PDT.

**Results** 18 patientes were included in the iTAAC group and 15 in control group. In both, foveal thickness and total macular volume (TMV) increase at visit 1 and decrease at visit 2 and 3, ever in a great magnitude in iTAAC group, with statistical significance in the TMV change at visit 3 (p-0,04). There is not significant change in visual acuity

**Conclusion** The combined treatment modify the pattern of foveal thickness change as compared with standard PDT therapy alone.

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## A pilot study investigating the safety and efficacy of a novel photodynamic agent, LS11

*HUSSAIN B, LIEW SHM, HAMILTON AMP, MARSHALL J*
*Ophthalmology Department, Rayne Institute, St Thomas' Hospital, London*

**Purpose** A pilot study investigating the safety and efficacy of a novel photodynamic agent, LS11, for the treatment of choroidal neovascularisation (CNV) secondary to age-related macular degeneration (AMD).

**Methods** A prospective study looking at 11 eyes of 11 patients with advanced AMD with no treatment alternatives. The main inclusion criterion was the presence of a disciform lesion with persistent leakage from CNV with a visual acuity (VA) of less than 6/60.Patients were administered 0.5mg/kg of LS11 after fundus fluorescein angiography (FFA) to delineate the lesion. An Oculight® laser was used to administer laser light of 664nm with the spot size varying between 1200 – 5000um depending on lesion size. The irradiance was 300 mW/cm2 and this was fractionated at fractions of 12 J/cm2. The primary end-point was 14 days with main outcome measures being closure of choroidal vessels on FFA, preservation of surrounding tissue, logMAR VA and any adverse events.

**Results** Eleven patients completed the study and all showed closure of CNV and reduction or cessation of leakage in treated areas. At 14 days follow-up, the VA of 3 patients improved by more than 0.1 logMAR, 7 were unchanged, and 1 deteriorated as a result of a branch retinal vein occlusion (BRVO).There were 4 adverse events: 2 patients suffered mild sunburn to the ears and top of the head, 1 patient had a small new retinal haemorrhage, and 1 had a BRVO.

**Conclusion** Photodynamic therapy using LS11 results in in closure of CNV and reduced leakage in advanced disciform lesions. Further research is needed to evaluate its effect on lesions at an earlier stage.

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## Age-related macular degeneration in African patients from D R Congo

*KAIMBO WA KAIMBO D (1), MISSOTTEN L (2)*
*(1) Department of Ophthalmology, University of Kinshasa, Kinshasa*
*(2) Department of Ophthalmology, catholic University of Leuven, Leuven*

**Purpose** To determine the frequency of age-related macular degeneration (AMD) in African patients from D R Congo.

**Methods** This was a retrospective study of all patients with AMD seen between January 1, 2002 and December 31, 2004. All patients had a dilated funduscopic examination including biomicroscopy. The diagnosis of AMD was made by clinical examination and, color fundus photography and/or fluorescein angiograms were obtained in all patients. AMD was defined as soft, distinct drusen with pigmentary abnormalities, within the macula in each eye. Additional to these findings, patients with dry AMD and geographic atrophy also showed a discrete area of retinal degeneration, with a sharp border and visible choroidal vessels with no evidence of choroidal neovascularization (CNV). Patients with CNV showed drusen and/or retinal pigment epithelial changes in at least one eye, in addition to CNV evidenced by subretinal macular hemorrhage, lipid deposits in the macula, fibrotic macular scarring, or retinal pigment epithelial detachment on fundus photographs.

**Results** AMD was found in 14 patients, giving a frequency of 0.2% (14 out of 5691 patients). There were seven female and seven male. The age ranged from 42 to 81 years (mean age, 63.14 years). Visual acuity ranged from counting fingers to 20/20. All patients showed dry AMD except one. Ocular associated conditions were found in six patients and included senile cataract (one patient), pseudoexfoliative glaucoma (one patient), hyperopia (two patients) and pterygium (two patients). Bilateral blindness was found in two patients.

**Conclusion** AMD seemed to be uncommon in African patients from D R Congo. Further studies are needed to confirm this founding.

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## Optical Coherence Tomography Findings in Welding Arc Maculopathy

*LUCAS RS (1), HARPER CA (2, 3), MCCOMBE MF (4), ZAMIR E (5)*
*(1) Ophthalmology, Royal Victorian Eye and Ear Hospital, Melbourne*
*(2) Centre for Eye Research Australia, Melbourne*
*(3) Medical Retina unit, Royal Victorian Eye and Ear Hospital, Melbourne*
*(4) Vitreoretinal unit, Royal Victorian Eye and Ear Hospital, Melbourne*
*(5) Ocular Immunology, Royal Victorian Eye and Ear Hospital, Melbourne*

**Purpose** To describe the optical coherence tomography (OCT) findings in two patients with welding arc maculopathy.

**Methods** Retrospective case series. In addition to examination by slit lamp biomicroscopy, colour fundus photographs and OCT images were obtained from patients with welding arc maculopathy.

**Results** Both patients had a history of bilateral decreased central vision after welding without appropriate eye protection. Yellowish foveal spots developed in the acute stage, evolving over several months into well demarcated lamellar foveal defects with surrounding retinal pigment epithelium changes. OCT showed interruption of the inner high reflective layer (HRL) corresponding to the level of the outer neurosensory retina. These appearances are similar to those seen in solar maculopathy.

**Conclusion** OCT shows disruption of the inner HRL in welding arc maculopathy. OCT can be a useful tool in confirming the diagnosis and understanding the pathogenesis of photic maculopathy.

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## Optical Coherent Tomography and Retinitis Pigmentosa -Therapeutic approach

*GONZALVEZ BG, HERA R, SARMENTO SA, MOUILLON MM*
*Ophtalmology- University hospital, Grenoble*

**Purpose** Optical Coherent Tomography (OCT) is an important imagery tool for the macular's edema evaluation in retinitis pigmentosa and its treatment. The manifestation of the retino vitreal's perimacular's tractions leads us to think that there is possibly a mechanical component in this edema physiopathology which incites us to evaluate a new therapeutic approach.

**Methods** Based on a representative observation of a 35 years-old woman, we demonstrated the retino vitreal interface quality and the macular edema OCT quantification in this macular distrophy. We have analyzed our results in a 5 years period after a medical treatment with carbonic anhydrase's inhibitor and intra vitreal injection of triamcinolone. We have also analyzed the direct correlation between the retina thickness and visual acuity.

**Results** The positive results of the medical treatment were restricted by its general tolerance. The intra vitreal injection of corticoids, which has already been described in the medical literature, has shown rapid and consistent effects. However, clinical and functional long-term comparisons between the treated and the non-treated eye are necessary.

**Conclusion** Does this findings allow us to consider taking aggressive gestures as the intra vitreal injection of corticoids or even the vitrectomy surgery, despite the phototrauma that may incur and its consequences for this pathology?

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## Central retinal thickness and volume after uncomplicated cataract surgery

*DEGENRING RF (1, 2), VEY S (1), KAMPPETER BA (1), BUDDE W (1), SAUDER G (1), JONAS JB (1)*
*(1) Faculty for Clinical Medicine Mannheim, Dept. of Ophthalmology, Mannheim*
*(2) Dept. of Ophthalmology, Cologne-Merheim*

**Purpose** To evaluate the influence of uncomplicated cataract surgery on the fovea.

**Methods** In a prospective study, 108 eyes were assessed by optical coherence tomography preoperatively and one day, one week and four weeks after uncomplicated phacoemulsification with endocapsular IOL implantation. The study included 24 eyes of diabetics. Eyes with preexisting macular edema oder eyes that developed cystoid macular edema within follow-up were excluded. Mean time of surgery was 11.5 ± 6.6 minutes.

**Results** Visual acuity increased from von 0.41 ± 0.15 to 0.81 ± 0.21 after four weeks ($p<0.001$) with non-diabetics showing a significant better results than diabetic subjects ($p=0.002$). Minimal foveal thickness increased from 183 ± 27 µm preoperatively to 191 ± 37 µm after four weeks ($p=0.001$). Diabetics had a tendency to more pronounced increase in retinal thickness than non-diabetics ($p=0.058$). One day and one week after surgery, minimal foveal thickness was not significantly higher than preoperatively. Foveal volume, however, showed a significant increase one week and four weeks after surgery ($p<0.001$), independently from presence of diabetes ($p=0.565$).

**Conclusion** Foveal thickness and foveal volume show a subclinical increase within 4 weeks after uncomplicated cataract surgery in diabetics and non-diabetics.

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## A peek at the OCT front intensity peak

*KESTING S, VECKENEER M, MISSOTTEN T*
*Rotterdam Eye Hospital, Rotterdam*

**Purpose** There is no consensus whether the front intensity peak in OCT originates from the anatomical presence of the Internal Limiting Membrane (ILM) or from the refractive index differences between the vitreous and the neuroretina. This front intensity peak corresponds to the first red-white line seen on commercial OCTs.The purpose of this study is to show the front intensity peak in OCT is present regardless of the ILM.

**Methods** In 13 patients, front intensity peaks, corrected for the baseline noise, were measured with Stratus OCT before and after vitrectomy with ILM peeling.

**Results** No significant difference (p=0.91) between the front peak intensities before and after surgery could be found. This demonstrates the front intensity peak is correlated with the refractive index difference between vitreous and retina.

**Conclusion** The front intensity peak in OCT corresponds to the vitreo-retinal interface and is independent of the ILM presence.

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## Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency in retinal vein occlusions

*PINNA A (1), CARRU C (2), SOLINAS G (3), DONAERA M (1), ZINELLU A (2), CARTA F (1)*
*(1) Institute of Ophthalmology, University of Sassari, 07100 Sassari, Italy*
*(2) Chair of Clinical Biochemistry, University of Sassari, 07100 Sassari, Italy*
*(3) Institute of Hygiene, University of Sassari, 07100 Sassari, Italy*

**Purpose** The reported incidence of G6PD deficiency in Sardina, Italy, ranges from 10% to 15%. Evidence indicates that patients with G6PD deficiency are protected against ischemic heart and cerebrovascular disease. The purpose of this study was to assess the incidence of G6PD deficiency in Sardinian patients with retinal vein occlusions and ascertain whether G6PD deficiency may have a protective effect against these vascular disorders.

**Methods** G6PD blood levels were measured in 169 consecutive patients (93 males, 76 females) with central retinal vein occlusion (CRVO) and in 170 consecutive patients (78 males, 92 females) with branch retinal vein occlusion (BRVO). 284 age-matched patients (146 males, 138 females) undergoing cataract surgery served as controls.

**Results** G6PD deficiency was found in 6 (3.55%) out of 169 patients with CRVO, in 9 (5.29%) out of 170 patients with BRVO, and in 25 (8.8%) out of 284 controls. Differences between CRVO patients and controls were statistically significant (OR: 0.38%, 95% CI: 0.12-0.98). Differences between BRVO patients and controls were not statistically significant (OR: 0.58%, 95% CI: 0.23-1.32).

**Conclusion** The incidence of G6PD deficiency in Sardinian patients with retinal vein occlusions was lower than expected. Results suggest that patients with G6PD deficiency have a significantly lower risk of developing CRVO.

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## Space-filling properties of retinal vessels and axial length

*PATTON N (1), MACGILLIVRAY T (2), DHILLON B (1)*
*(1) Ophthalmology, Princess Alexandra Eye Pavilion*
*(2) Welcome Trust Clinical Research Facility, Edinburgh*

**Purpose** To explore the relationship between parameters reflecting the space-filling properties of retinal vessels and axial length.

**Methods** 52 pseudophakic patients with pre-existing reliable axial length measurements were recruited. All patients underwent 35mm fundal colour photography (50 degree field, centred on the disc) and images were digitalised at 3200 dpi. Using a customised image processing package written in the Matlab® environment, image analysis was performed and quantitative dimensionless measurements that reflected the space-filling properties of the retinal vasculature were performed, including retinal angles at arteriolar bifurcations, arterial tortuosity , and the fractal dimension of the arteriovenous retinal vascular tree. A novel user-friendly technique for the simple calculation of arteriolar tortuosity is presented. Fractal dimension of the arteriovenous tree was calculated based on a skeletonised binary image, using the box counting technique.

**Results** Mean arterial vascular tortuosity related to the angles at arteriolar vessel bifurcations(R=0.36, p=0.009) and also correlated with the fractal dimension of the arteriovenous fractal dimension (R=0.284, p=0.046). Axial length does not relate to either the fractal dimension of the arteriovenous tree (R=-0.28, p=0.846), the angles at vessel bifurcations (R=0.004, p=0.98) or arteriolar tortuosity(R=-0.189, p=0.180).

**Conclusion** Axial length does not affect either angles at vessel bifurcations, tortuosity of vessels or the fractality of the arteriovenous network. Retinal arterial tortuosity relates to the angles at which vessels bifurcate at vascular junctions, possibly reflecting a greater likelihood for obtuse angles from circuitous vessel paths.

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## Central retinal artery occlusion with simultaneous contralateral central retinal vein occlusion

*CHRONI FC (1), SKINNER KC (1), GREGORY PT (1), MERRICK CD (1), LAPPAS I (2)*
*(1) Conquest Hospital, St. Leonards-On-Sea*
*(2) Evagelismos Hospital, Athens*

**Purpose** To report an unusual case of CRAO with contralateral CRVO.

**Methods** Case report

**Results** A 75-year old male presented with decreased vision in both eyes.Visual acuity of the RE was LP and had a marked right RAPD.VA of the LE was 6/60.Fundus biomicroscopy revealed CRAO in the RE and CRVO in the LE.Digital fundoscopy confirmed the diagnosis.Digital massage and paracentesis was performed.IOP lowering drugs were used.VA of the RE didn't recover.VAL improved to 6/24.Battery of tests came back normal apart from high MCV. He is still under follow up at the hospital.

**Conclusion** Aetiology of condition is discussed.There have been reported several conditions associated with ipsilateral combined CRAO and CRVO such as giant cell arteritis,collagen vascular disease other than GCA, hypercoagulation disorders, migraine, syphilis.To the best of our knowledge this is the first report of CRAO combined with contralateral CRVO.

## ▪ 437
## Roth's spots and BRAO simultaneously in same patient

*SKINNER KC, CHRONI FC, MERRICK CD*
*Conquest Hospital, St-Leonards-on-Sea*

**Purpose** To report a patient who was diagnosed with staph aureus endocarditis and subsequently developed Roth's spots and BRAO in the same eye.

**Methods** Case report

**Results** A 76 year gentlemen was admitted as an emergency under the medical team with sepsis. He was subsequently found to have a new onset murmur and grew Staph aureus in blood cultures. An echo showed tufts of vegetation on the valve and a diagonosis of sub acute endocarditis was made. He was treated as such with intravenous antibiotics. During the course of his treatment to substantiate this diagnosis he was referred to the eye department to look for the prsence of Roth's spots. On fundal exam he was found to have Roth's spots as well as a fresh BRAO in the same eye with no effect on his vision.

**Conclusion** To date there has not been a case reported of simultaneous BRAO and Roth's spots in the same patient. It is well reported that Roth's spots occur with endocarditis and the presence of these are often used to help with diagnosis as this can be difficult. The occurence of BRAO may also aid diagnosis but may be overlooked as the signs can be more subtle. This case therefore highlights the importance of not only looking for Roths spots but also to look carefully for signs of arterial occlusion.

## ▪ 438
## Quinine: intoxication or vascular spasm?

*NEUDECK M, KLOPFER M, LANZL I*
*Augenklinik, Klinikum rechts der Isar, Munich*

**Purpose** Quinine is a long known drug used for malaria and sometimes prescribed for muscular cramps. We present a case of quinine overdose, discussing the possible mechanism of retinal affection.

**Methods** A 52 year old woman overdosed on her quinine prescription for muscular cramps in a suicidal attempt.The intake was 3 grams with 400 mg being the dayly dose for cramps and up to 1.200 mg for malaria cases. Few hours later her visual acuity was reduced bilaterally to no light perception.

**Results** The fundus examination at first presentation showed spastic thinning of retinal arteries, the optic disc appeared normal. Electrophysiology revealed massive disturbances in the retinal function, pigment epithelial function as well as ganglion cell function two days after ingestion. Light perception was regained three days after ingestion. The final visual acuity reached 20/20 bilaterally 1 month after the insult, with massive concentric visual field loss to the central 15°. Retinal vessels seemed normal and the optic disc showed increasing pallor.

**Conclusion** We found a massive disturbance in first and second neuron function immediately after the insult by ERG. Whether this is due to the observed vascular spasms inducing a neuronal ischemia or a direct neurotoxic mechanism remains unclear. The EOG being reduced after the insult rather hints to a direct neurotoxic effect since the pigment epithelium is rather resistant to short term ischemic insults. We would like to alert prescribing physicians that the therapeutic width of quinine is rather narrow and that suicidal patients should not be prescribed the drug for cramps.

## ▪ 439
## An illuminated scleral indenter in the teaching of indentation techniques to trainee ophthalomologists

*DWARIKA D (1), BHOLA RM (2), SCOTT RAH (1)*
*(1) Birmingham and Midland Eye Centre, Birmingham*
*(2) Academic Unit of Ophthalmology and Orthoptics, The University of Sheffield, Royal Hallamshire Hospital, Sheffield, Sheffield*

**Purpose** To test a novel scleral indenter (depressor) design in the teaching of scleral indentation technique to trainee ophthalmologists.

**Methods** A scleral indenter with an illuminated area on the depressing tip was developed. A white light emitting diode (LED) delivered the illumination via a fibre optic link from the rod like handle. A pilot study was conducted comparing the ease of identifying the scleral indent with the illuminated indenter and the standard Schoket and Schepen indenters.

**Results** All subjects concluded identification of the indent and also control of the dynamic process of indentation was easier and more reproducible with the illuminated instrument.

**Conclusion** The pilot study shows that the illuminated indenter makes learning indentation easier for the trainee. This is invaluable for ophthalmic training as scleral indentation is one of the most difficult clinical ophthalmic skills to acquire.

## ▪ 440
## The ERG assessment of rabbit retina vulnerability to intravitreal triamcinolone

*ZUEVA M (1), TSAPENKO I (1), NEROEV V (2), TANKOVSKY V (2), MASLOVA-KHOROSHILOVA I (3), MIZEROVA O (2)*
*(1) Clinical Physiology of Vision (Moscow Helmholtz Research Institute of Eye Diseases), Moscow*
*(2) Pathology of Retina and Optic Nerve (Moscow Helmholtz Research Institute of Eye Diseases), Moscow*
*(3) Pathohystology (Moscow Helmholtz Research Institute of Eye Diseases), Moscow*

**Purpose** To evaluate rabbit retina structures more vulnerable functionally to intravitreal triamcinolone acetonide (TA).

**Methods** The study was carried out in 8 rabbits who received the single intravitreal injection of 8mg or 6mg of TA to the right eyes. The BSS was injected to the left eyes. The single-flash ERG and 4-32 Hz flicker ERGs were registered prior to and 1-3 days, 2-3 weeks, 1, 1.5, 6 months after the TA injection. The glial index Kg was calculated [Zueva et al., 2004]. The histopathologic examination was carried out at the end of the follow up.

**Results** At the lower dose of TA, the single-flash and flicker ERGs amplitudes showed the initial decrease on days 1-3 and then significant increase on 2-3w – 6m after the injection. At the higher dose, we observed two different retina reactions: (I) All biopotentials showed the initial increase on weeks 2-3 and then the decrease in amplitudes, which did not restore at the end of the follow up. (II) amplitudes of all ERGs increased to norm values or more. After the 6 mg TA, a transient increase in Muller cells (MC) glial index Kg we revealed on days 1-3; it was normalized after 2-3 weeks. The higher doses resulted in a prolonged increase of MCs' activity in group I or in a transient decrease in group II. Changes in flicker ERGs between 8-12Hz and in the a-wave of a single-flash ERG were more significant. There were no TA-related toxic effects evident on clinical or histopathologic examination of eyes.

**Conclusion** Intravitreal TA showed no evidence of drug toxicity. However, the retina function was vulnerable to TA and the 8 mg dose should not be considered potentially safe. Our findings suggest a primary TA interference in photoreceptor's and MC's function.

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## Correlation between stage of retinopathy of prematurity and optic disc morphology

*OBERACHER-VELTEN I, ELFLEIN H, LORENZ B*
*Paediatric Ophthalmology, University Eye Hospital, Regensburg*

**Purpose** The aim of the present study was to investigate the influence of stage of retinopathy of prematurity (ROP) on optic disc morphology.

**Methods** Widefield images (120°, Retcam system) of 114 eyes of 57 children with ROP (mean gestational age at birth 27,2 weeks, range 24 to 30 weeks) were analyzed regarding optic disc shape, localization of the vessel trunk, and the proportion between the horizontal nasal and temporal neuroretinal width. Classification of ROP was performed according to international standards (ICROP).

**Results** In all optic discs, the vertical disc diameter was higher than the horizontal diameter. There was a tendency towards a higher ratio of the vertical to the horizontal optic disc diameter in eyes with ROP compared with eyes without ROP. Eyes with ROP stage 3 had a significantly higher ratop of vertical to horizontal optic disc diameter than eyes with ROP stage 1 ($p < 0.05$). There was neither a statistically significant difference between the ratio of vertical and horizontal optic disc diameter in eyes with ROP stage 1 and ROP stage 2 nor between eyes with ROP stage 2 and 3. Regarding all eyes included, the horizontal nasal neuroretinal rim was significantly wider than the temporal rim ($p < 0.05$). There was a tendency towards a lower ratio of temporal to nasal neuroretinal rim width in infants with ROP compared to babies without ROP.

**Conclusion** Optic disc morphology in prematures seems to be dependent on the presence and stage of ROP. The lower ratio of temporal to nasal neuroretinal rim width in infants with ROP might be due to the often reduced macular development in these children. Further studies have to show the value of optic disc morphology as predictive factor for visual outcome in premature infants.

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## Ischemic Retinopathy with stem cell auto graft?

*RAVINDRANATHAN R, TAHHAN M*

**Purpose** To report a case of ischemic retinopathy in a patient with stem cell autograft.

**Methods** Retrospective case note review.

**Results** A 45 years old man had a cervical mediastinotomy and the histological diagnosis was follicular non-Hodgkin's lymphoma. He was started on chemotherapy, which included cyclophosphamide. After total body irradiation, he was given a stem cell dose of CD34cells. His haemoglobin was regularly monitored and was 13.5 g/dl. Platelet count was 200-180x10/l. His blood pressure was in the range of 100/65-140/95. He was started on alpha interferon. Two months later, the patient started complaining of tiredness and lethargy. Haemoglobin range was 8.1-10.9 g/dl and the platelet count came down to 87x 10/l and blood transfusion was given.As the symptoms of tiredness increased, alpha interferon treatment was stopped. A few weeks later he developed blurring of vision and was referred to an ophthalmologist whose examination revealed, visual acuity 6/9 RE 6/6 LE, no afferent papillary defect, intraocular pressure 18mmhg in both eyes. Anterior segment was normal. Fundus examination showed extensive posterior pole nerve fibre layer haemorrhages and cotton wool spots, the optic disc was not swollen (figure1). Haemoglobin was 10.8-12.5g/dl, platelet count was 214-250x10/l and blood pressure was 190/110. Antihypertensive treatment was started.Subsequent review by the ophthalmologist, a month later, showed visual acuity 6/6 both eyes. Examination of the anterior segment and posterior segment was normal.

**Conclusion** Cyclophosphamide can cause retinal haemorrhages, but ischemic retinopathy with cotton wool spot is said not to occur.Radiation is a potent cause of haemorrhages ischemic retinopathy and the lowest dose causing radiation retinopathy is 30Gy. Anaemia of less than 8g/dl and thrombocytopaenia with platelet count below 50x10/l can give rise to retinal haemorrhage. Our patient's haemoglobin and platelet count never fell to these levels.Hypertension can cause an ischemic haemorrhagic retinopathy. Our patient's blood pressure was not excessively high, and no signs of hypertensive retinopathy were noted.Interferon has been documented as causing ischemic haemorrhagic retinopathy at dose more than 880 million units and our patient received 189 million units. Finally, ischemic retinopathy is a well-characterised complication of bone marrow transplantation. Our patient received stem cells autograft transplant which has not been reported it before as one of the causes of ischemic retinopathy.

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## Microperinetry Evaluation in Serpiginous Choroidopathy

*PLAZAS A (1), RUIZ-LAPUENTE C J (2), DEL ESTAD A (2), RUEDA T (2)*
*(1) Department: Ophtalmology (HH.UU. Virgen del Rocío), Sevilla*
*(2) Ophtalmology (HH.UU. Virgen del Rocío), Sevilla*

**Purpose** To investigate the effects of serpiginous choroidopathy on macular fundus parameters quantified with a liquid crystal display microperimetry.

**Methods** Case report: A 50-year-old man undergoing serpiginous choroidopathy, presented with a history of decrease vision in both eyes. On examination, no reduction in visual acuty was objectively detected. Fundus evaluation revealed characteristic lesions of his basic pathology, but no signs of activity were found neither by funduscopy nor angiofluorescein study. A microperimetry examination was performed to assess the function of the macula and central visual field as well as the location and stability of fixation during a follow-up period of four months.

**Results** Microperimetry evaluation didn´t show significative changes in macular sensitivity or fixation.

**Conclusion** Microperimetry may be an useful tool in evaluating macular diseases by quantifying the macular function in a more exactly way and therefore by explaining the influence of theses pathologies in patient´s life.

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## Effects of patent blue on human retinal function

*LÜKE C (1, 2), LÜKE M (3, 4), SICKEL W (5, 2), SCHNEIDER T (5, 2),*
*MARZELLA G (6), KIRCHHOF B (1, 2)*
*(1) Center of Ophthalmology, Cologne*
*(2) University of Cologne, Germany*
*(3) Department of Ophthalmology, Tübingen*
*(4) University of Tübingen, Germany*
*(5) Institute of Neurophysiology, Cologne*
*(6) Augenheilkunde am Rheincenter, Cologne*

**Purpose** Dye solutions for intraoperative staining of epiretinal membranes and the internal limiting membrane improve the visualisation of these thin structures and facilitate their removal. Lately, the application of patent blue for staining of the internal limiting membrane has been proposed as an alternative to the standard procedures during macular hole surgery. In the present study we investigated the effects of patent blue 0.48% on human retinal function.

**Methods** An isolated human retina preparation was perfused with a standard solution and the electroretinogram (ERG) was recorded repeatedly. After recording of stable ERG amplitudes the nutrient solution was substituted by a commercially available patent blue solution. The duration of retinal exposure to the dye solution was varied between 15 seconds and 4 minutes. Thereupon, the preparation was reperfused with standard solution for 2 hours. The percentage of b-wave reduction after exposition was calculated.

**Results** No effects on the human ERG were seen after 15 and 30 seconds of dye application. Reversible reductions of the b-wave amplitude were found for an exposure period of 60 and 120 seconds, respectively. After 4 minutes of patent blue application a persistant b-wave amplitude reduction by 40% was found.

**Conclusion** Patent blue affects human retinal function when applied for at least one minute. However, no irreversible effects on the human ERG were seen even after two minutes of retinal exposure to patent blue. Thus, toxic effects on retinal function after intraoperative short-term application of patent blue 0.48% appear unlikely to occur.

## ▪ 445 / 4136
## Multispectral Fundus Analysis

*CALCAGNI A (1, 2), STYLES IB (1), CLARIDGE E (1), ORIHUELA-ESPINA F (1), GIBSON JM (2)*
*(1) University of Birmingham - School of Computer Science, Birmingham*
*(2) Birmingham and Midland Eye Centre, Birmingham*

**Purpose** To generate a reflectance model of the fundus that allows an accurate non-invasive quantification of blood and pigments.

**Methods** A Monte Carlo simulation was used to produce a mathematical model of light interaction with the fundus at different wavelengths. The model predictions were compared with fundus images from normal volunteers in several spectral bands (peaks at 507, 525, 552, 585, 596 and 611nm). The model was then used to calculate the concentration and distribution of the known absorbing components of the fundus.

**Results** The shape of the statistical distribution of the image data generally corresponded to that of the model data; the model however appears to overestimate the reflectance of the fundus in the longer wavelength region.As the absorption by xanthophyll has no significant effect on light transport above 534nm, its distribution in the fundus was quantified: the wavelengths where both shape and distribution of image and model data matched (<553nm) were used to train a neural network which was then applied to every point in the image data. The xanthophyll distribution thus found was in agreement with published literature data in normal subjects.

**Conclusion** We have developed a method for optimising multi-spectral imaging of the fundus and a computer image analysis capable of estimating information about the structure and properties of the fundus. The technique successfully calculates the distribution of xanthophyll in the fundus of healthy volunteers. Further improvement of the model is required to allow the deduction of other parameters from images; investigations in known pathology models are also necessary to establish if this method is of clinical use in detecting early chroido-retinopathies, hence providing a useful screening and diagnostic tool.

## ▪ 446 / 4137
## Zinc in Drusen

*PETO T (1), LENGYEL I (2, 3), FREDERICKSON C (4), VAN KUIJK E (5)*
*(1) Moorfields Eye Hospital, London*
*(2) Institute of Ophthalmology, London*
*(3) Biological Research Centre, Szeged*
*(4) Neurobiotex, Galveston*
*(5) Ophthalmology and Visual Science, Galveston*

**Purpose** Abnormal zinc metabolism has been described in retinal disorders such as age-related macular degeneration (AMD). One of the hallmarks of AMD is the appearance of drusen both peripherally and in the macula. As there is no conclusive data on Zinc in Drusen, our present study concentrated on demonstrating the presence of readily available or chelatable zinc in drusen in human donor eyes with AMD.

**Methods** Frozen donor eyes from the Montana Eye Bank were used and were photographed before dissection. Using a trephine, 6 mm diameter regions of the peripheral and central retinae were dissected. Following defrosting, the neuronal retina and the retinal pigment epithelial cells were removed to expose the underlying Bruch's membrane with or without drusen. Paired samples from individual eyes were treated in two ways. One of the samples was immersed in phosphate buffer saline (PBS) that contained 100 µM of the zinc specific chelator TPEN and the second sample was in PBS only overnight before 10 µM ZP1 (a zinc specific fluorescence probe) in PBS was applied to both samples for 5 min. Excess ZP1 was removed by rinsing the samples in fresh PBS and the changes in fluorescence was viewed by a NIKON fluorescence microscope (excitation: 460-500 nm; emission: 530-560 nm).

**Results** The samples that were treated with PBS only there was a robust increase in fluorescence in the presence of ZP1, but in the TPEN treated samples the increase in fluorescence was not detectable, signifying the presence of chelatable zinc. Long incubation with ZP1 resulted in punctate staining within drusen that appears to be located within caveoli and certain internal structures in some drusen.

**Conclusion** Therefore, chelatable zinc is present in drusen both in the periphery and in the macula and may play a role in the pathogenesis of AMD.

## ▪ 447 / 4236
## Morphological Features of Classic and Occult subfoveal Choroidal Neovascular Membrane using OCT -An aid to diagnosis

*ADEWOYIN TA, KHAN J, CHONG NHV*
*Laser and Retinal Research Unit, King's College Hospital, London*

**Purpose** To assess the morphological differences of choroidal neovascular membrane on optical coherence tomography (OCT) in classic and occult CNV.

**Methods** Consecutive case series of 57 eyes of 57 patients diagnosed wih acute CNV undrwent OCT-3 examination, followed by fluorescein angiogram.Fluorescein angiograms were reported using TAP guidelines.The OCT images were assessed based on the presence of intraretinal fluid and subretinal fluid, the presence of a discrete subretinal lesion and the presence of pigment epithelial detachment.

**Results** Regression analysis suggested that classic lesions are more likley to have a discrete sub retinal lesion and intraretinal fluid, whereas occult CNV are more likely to have fluid in the sub retinal or RPE space. Using these characteristics,96% of classic lesions and 70% of occult lesions had shown the above mentioned respective features.

**Conclusion** Optical coherence tomography is a non invasive powerful tool. Morphological differences between classic and occult neovascular lesions enable their diagnosis using OCT with reasonable accuracy compared to the gold standard of fluorescein angiogram.

## ▪ 448 / 4237
## Photodynamic therapy versus surgical removal from choroidal neovascularisation in high myopia

*HERA R, VINH V, GONZALVEZ B, MILLET JY, ROMANET JP, MOUILLON MM*
*Ophthalmology, Grenoble*

**Purpose** Several treatments of choroidal neovascularization (CNV) in pathologic myopia are proposed today. We compared, by a retrospective analysis, the visual outcome at 12 and 24 months between patients who underwent surgical removal of CNV and patients treated by photodynamic therapy(PTD).

**Methods** We reviewed, after two years of follow up, clinical and angiographic findings of 25 patients with severe myopia and subfoveal classic CNV. The patient population was divised in 2 groups:11 patients treated by PTD and 14 patients treated by surgery.

**Results** An intermediary analysis was performed at 12 months and showed a significant visual acuity benefit for the group treated by surgery. The final results at 24 years showed no significant diffference in the visual outcome between the two groups.

**Conclusion** Even if the surgical removal is more benefic for the visual outcome in the first year, the final visual acuity is quite similar for the two groups after two years of follow up. In the light of this results we conclude that in CNV of pathologic myopia, PTD is preferable to surgical removal because is more safe.

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## Audit of diabetic referrals – Rationalising patient referrals via a three-tier system

*FLETCHER EC, ADEWOYIN TA, CHONG NHV*
*Kings College Hospital, London*

**Purpose** Screening services for diabetic retinopathy provide an invaluable facility aiming to ensure appropriate referral of patients to the retina specialist centres. We would like to describe a modified and enhanced service running in King's College Hospital, which enables suitable and timely referrals.

**Methods** Annual retinal screening is performed using retinal photography by the Diabetic Eye Complication Screening Service. A trained level 1 screener / photographer interprets the photographs on the spot to assess whether the images were normal and acceptable. Abnormal images are then passed on to the level 2 screener for referral decision. The treating retinal specialist can assess the referred images using the same software. If referral is not deemed necessary at this stage then advice regarding further screening is given and the patient informed of the outcome.

**Results** Of the 2260 patients screened between Nov 2004 to April 2005, referral was requested on 186 (8.2 %). Of those, 94 (50.5 %) were accepted for examination in the retina unit for possible treatment. The most common reasons that further assessment were not required were early maculopathy without clinically significant macular oedema, fundal lesions of no consequence and previously treated but no active maculopathy.

**Conclusion** Diabetic retinopathy screening has already been proven to provide both a sensitive and specific method for detection of sight threatening diabetic retinopathy. This three tier method reduces referrals by ~50% and thus forms a streamlined service ensuring that busy retinal clinics are used effectively and provides an improved service for patients who can remain under close observation in their local area.

■ **450 / 4336**

## Detection of associated ocular lesions during screening of diabetic retinopathy

*DEB-JOARDAR N (1), THURET G (1), GERMAIN N (2), GARCIN AF (1), MANOLI P (1), GAVET Y (3), ESTOUR B (2), GAIN P (1)*
*(1) Department of Ophthalmology, University Hospital, Saint-Etienne*
*(2) Department of Endocrinology, university hospital, Saint-Etienne*
*(3) Ecole nationale supérieure des Mines, Saint-Etienne*

**Purpose** Screening for diabetic retinopathy (DR) is insufficient in France mainly due to an increased incidence of diabetes and lack of enough ophthalmologists. In this setting, digital camera provides a sensitive and cost-effective option. Apart from DR, this allowed to detect other coexisting pathologies

**Methods** We prospectively recorded the fundus photos of 1153 consecutive patients during 1 year. Patients underwent five-field (45°) imaging through pharmacologically dilated pupils (Topcon TRC NW6S) followed by specialist interpretation. Patients were interrogated for antecedent history and referred for further evaluation

**Results** The patients (578 males, 575 females) aged 57±16 years had a 14±11 years evolution of diabetes. Coexisting pathologies were recognized in 612 patients (53%). The most common associated lesions were hypertensive retinopathy (n=205, 18%), significant cataract (n=176, 15%), age-related macular degeneration (ARMD) (n=66, 6%) and optic nerve excavation (n=33, 3%). Twenty-three patients (2%) had previously undiagnosed sight-threatening lesions: ARMD (n=4), retinal vein occlusions (n=4), florid hypertensive retinopathy (n=3), macroaneurysms (n=2), macular hole (n=1), rhegmatogeneous retinal detachment (n=1), retinitis pigmentosa (n=1) and choroidal mass lesion (n=1). Significant optic nerve lesions detected were papilloedema due to intracranial metastasis (n=1), optic atrophy (n=2, pituitary tumour, nonarteritic neuropathy) and glaucomatous cupping (n=1)

**Conclusion** Multiple potentially sight-threatening lesions apart from DR were detected during routine screening. Quality interpretation and timely referrals may help to prevent possible visual loss

■ **451 / 4337**

## A two-year prospective study of mild nonproliferative retinopathy in subjects with diabetes type 2 undergoing intensive oral tritherapy. Progression of retinopathy under stabilized metabolic control

*DUARTE L (1), RIBEIRO ML (2), NUNES S (2), LOBO C (2, 3), BERNARDES R (2), GERALDES E (4), FARIA DE ABREU JR (3), CUNHA-VAZ JG (2, 3)*
*(1) AIBILI – Association for Innovation and Biomedical Research on Light and Image, Coimbra*
*(2) AIBILI, Coimbra*
*(3) Department of Ophthalmology, University Hospital, Coimbra*
*(4) Department of Endocrinology, University Hospital, Coimbra*

**Purpose** To evaluate progression of mild nonproliferative retinopathy in subjects with diabetes type 2 under stabilized metabolic control

**Methods** Forty-five type 2 diabetic patients with mild nonproliferative retinopathy were treated with an association of three hypoglycemic agents (biguanide, alpha-glucosidase inhibitor, sulphonylurea) to optimize their metabolic control. Patients were followed-up prospectively for a two-year period.

**Results** Metabolic control remained stabilized with an overall decrease in HbA1C levels from 7.5% ± 1.2% to 7.4% ± 1.6%. The number of red dots in the fundus of the eye (microaneurysms + hemorrhages), however, showed significant overall increases at every visit. The rates of progression of number of red dots per year varied among patients, showing a correlation with the rate of progression before enrollment ($p = 0.007$) and increasing with HbA1C levels > 7.1% ($p < 0.001$).The number of red dots correlated with changes in the permeability of the blood-retinal barrier (BRB) and alterations in the foveal avascular zone (FAZ) when present

**Conclusion** Progression of retinopathy in type 2 diabetes during a two-year follow-up period, under stabilized metabolic control, varies among patients. Progression is more marked in eyes with more pathology at baseline and in patients presenting higher HbA1C levels.

■ **452 / 4338**

## Subtenon triamcinolone acetonide to treat diabetic macular edema

*CELLINI M, ZAMPARINI E, BRAVETTI GO, BELLUSCI C, CAMPOS EC*
*Department of Surgery and Transplant "A. Valsalva", Bologna*

**Purpose** To evaluate the efficacy of Triamcinolone Acetonide subtenon injections in the treatment of diabetic macular edema.

**Methods** We selected 11 patients, 8 of which were male, and 3 of which were female, ages ranging between 61 and 74 years old (average 68.3). The patients were all affected by diabetes type II and they presented macular edema previously diagnosed with fluorescence angiography. The patients' visual acuity was evaluated utilizing the ETDRS logarithm (logMAR), the measurement of intraocular pressure (IOP), and a biomicroscopic examination of both anterior and posterior segments of the eyes. Subsequently, an Optical Coherence Tomography (OCT) was performed using an OCT3 (Zeiss-Humphrey, Dublin, CA).After a topical 0.4% oxybuprocaine anesthesia, the patients were given a 40 mg peribulbar inferotemporal subtenon injection of Triamcinolone Acetonide. Each patient was given three injections; each injection separated by 30 days. After each injection, the patients were prescribed a topical antibiotic treatment for three consecutive days.

**Results** Visual acuity before cortisonic treatment was 0.464+0.112 log with a 16.182+1.4011 mm/Hg. A month after the beginning of the treatment, visual acuity was 0.118+0.125 log ($p<0.001$) while the IOP was 16.727+1.191 mm/Hg ($p<0.147$). Twelve months after the end of the treatment, we observed a stabilization of visual acuity and IOP. After three months of treatment, the condition was successfully cured. The OCT results were also stable a year after the treatment.

**Conclusion** Utilizing Triamcinolone Acetonide with a peribulbar subtenon injection showed to be an effective solution for diabetic macular edema without showing signs of the complications.

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## Comparison of measurements with OCT2 and OCT3 in patients with achromatopsia

*VARSANYI B, SOMFAI GM, LESCH B, FARKAS A*
*Semmelweis University, Department of Ophthalmology, Budapest*

**Purpose** To examine in vivo the anatomical structure of the retina of patients with achromatopsia, and compare the results obtained with different types of measurements with OCT2 and OCT3 devices.

**Methods** 11 eyes of 6 patients with congenital achromatopsia and 18 eyes of 9 control subjects were examined by optical coherence tomography. 6 mm radial scans were taken of the macula, centred on the fovea. The thickness of the neuroretina was measured manually, and also automatically with the built-in software in 5 sections: at the foveola, ±1.5, and ±3 mm away. Total macular volume (TMV) and the retinal thickness in the 9 ETDRS-regions were calculated also by the built-in software of the device.

**Results** Regarding the TMV and the thickness of the 9 ETDRS regions, there was no difference between the OCT2 and OCT3 data: both showed significant reductions in the TMV and in the retinal thickness of the patients with achromatopsia. We found significant difference between the "manual" and the "automatic" measurements in all the 5 sections of the retina, both in the control and in the patient group. With all measurement methods, the retina of the achromatopsia patients was found significantly thinner than that of control subjects, except for the foveola.

**Conclusion** The overall structure of the macula in achromats differs from that in normal subjects. Our measurements show the thinning of the macula. The higher resolution of the OCT3 helps identify the anatomical structure better. This is crucial in the foveola, which is the most highlighted part of the retina of the achromats, since this is the place where cones - influenced by the disease - abound.

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## Preventive effect of pigmentation on ERG responsiveness in P23H rats

*SAUVÉ Y (1), PINILLA I (2, 3), CUENCA N (4, 5), LUND RD (5)*
*(1) Ophthalmology, Edmonton*
*(2) Hospital Universitario Miguel Servet, Zaragoza*
*(3) Moran Eye Center, Salt LakeCity*
*(4) Biotechnology, Alicante*
*(5) Moran Eye Center, Salt Lake City*

**Purpose** The albino P23H transgenic rat is an extensively studied model of autosomal dominant retinitis pigmentosa (RP). There is indication that albinism itself might exacerbate the disease. We examined the potential preventive effect of pigmentation on functional deterioration using the electroretinogram (ERG).

**Methods** Pigmented rats heterozygous for the P23H mutation in the rhodopsin gene were generated by breeding normal pigmented Long Evans (LE) rats with homozygous albino P23H rats. Both LE (serving as controls) and heterozygous P23H pigmented transgenic rats aged from P18 to P180 were studied. Corneal ERGs were recorded under scotopic and photopic conditions.

**Results** While both rod- and cone-driven ERG responses increased in maximal b-wave amplitudes from P18 to P29, only cone-driven b-wave responses reached developmental maturity in P23H rats (by P29 compared with P21 in LE rats). Flicker fusions were also comparable at P29 (42 Hz). Although double flash-isolated rod-driven responses were already affected at P29 (a- and b-waves peaking to 203 and 564µV, compared with 610 and 998µV in LE rats) they were still elicited at P180 (a- and b-wave maximal amplitudes of 60 and 211µV). The rod-driven contribution to mixed scotopic b-waves was slightly lower in P23H (70%) compared with LE (79%) rats, but remained at 58% by P180. Photopic responses revealed a slow deterioration after P29. By P180, maximal photopic b-waves were still half of the amplitudes in LE rats, and flicker fusion at 31Hz.

**Conclusion** The deterioration in ERG responsiveness in pigmented P23H rats is slower than previously reported for the albino P23H rats; supporting that albinism itself might indeed exacerbate RP-related defects. Support: NIH (EY14038), FIS (PI042399), ONCE, Fundaluce.

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## Quantitative Phenotyping of Stargardt Disease with Novel Psychophysical Perimetric Techniques and Multifocal Electrophysiology

*MAIA-LOPES S (1), SILVA MF (1), FARIA PA (1, 2), SILVA E (1, 2), REIS A (1, 2), CASTELO-BRANCO M (1)*
*(1) IBILI-Center for Ophthalmology, Faculty of Medicine, Coimbra*
*(2) Coimbra University Hospital, Coimbra*

**Purpose** To study the psychophysical phenotype and the multifocal ERG (mfERG) of 13 families with Stargardt's Disease (SD).

**Methods** Sixteen SD patients, 12 relatives (normal ophthalmologic exam) and 25 controls were studied. Chromatic performance was evaluated with an anomaloscope (IF-2) and a modified version of the Cambridge Colour Test. Subjects also underwent two different custom-made perimetries to obtain contrast threshold measures in 17 locations. The first isolated the magnocellular pathway and the second had a stronger parvocellular bias. MfERGs were also obtained.

**Results** Chromatic performance was impaired in SD patients. Magnocellular perimetry revealed lower contrast sensitivity in patients in comparison both to their relatives and controls. Interestingly, patients were significantly impaired across the whole visual field. SD relatives have a very significant better performance than SD patients for all the tested zones. However, an unexpected impairment was found in SD relatives as compared to controls. Parvo-biased perimetry showed similar results. MfERGs revealed significant reduced N1 and P1 amplitudes in SD patients and their relatives, not exclusive to the macula. N1 and P1 latency measures showed small but significant delays in both waves only in patients.

**Conclusion** Magno and parvocellular functions are impaired in SD patients across the whole visual field. It is worth noting that SD relatives show a subtle pattern of impairment, particularly regarding parvocellular function. These findings are consistent with the reduced amplitudes found in mfERGs, where the impairment of SD relatives is even more evident.

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## Distinct response topographies observed with SLO-based and conventional multifocal ERG

*SEBASTIÃO A (1, 2), MAIA-LOPES S (1), NUNES S (2), REIS A (3), CASTELO-BRANCO M (3)*
*(1) IBILI - Institute of Biomedical Research in Light an Image, Coimbra*
*(2) AIBILI - Association for Innovation and Biomedical Research on Light and Image, Coimbra*
*(3) IBILI–Center Ophthalmology, University Coimbra, Coimbra*

**Purpose** To compare response topographies obtained with SLO-based and conventional mfERG.

**Methods** To record mfERG we used a SLO-based system (RETIScan connected to a HRA Heidelberg SLO) with a blue laser (488nm) and a conventional RETIScan system whose monitor displayed stimuli modulated both along achromatic and chromatic axes. The stimuli consisted of 19 hexagon arrays covering a visual field of 15º and 30º for the SLO-based and the standard systems, respectively. MfERG recordings were taken using DTL fiber electrodes and responses were compared for the central 15º. Fixation was continuously monitored with both methods. Kodak-Wratten neutral density filters with different attenuation properties were used to study the effect of light intensity on the amplitude of SLO-mfERG responses.

**Results** P1 mean amplitudes were significantly reduced in the central retina using 488nm stimuli, as well as for a comparable chromatic modulation in the monitor. A pattern of significant up-down visual field asymmetry was observed in P1 amplitudes obtained with SLO system, as well as a weaker naso-temporal asymmetry. Asymmetries were detectable under different light intensities. Up-down asymmetries were not evident for achromatic stimuli presented in the monitor display, in contrast to the anisotropy observed under chromatic modulation.

**Conclusion** Distinct response topographies were revealed under different stimulus conditions with SLO-based and standard mfERG. The lower central response cannot be simply explained by hexagon size differences and may be related to differential temporal and spatial response properties of different cone populations. The up-down asymmetry is probably related to anisotropies in the S-cone pathway functional anatomy.

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## Visual Information Processing from extracellular recording in Lateral Geniculate Nucleus (LGN) of the rat

*CEPEDA IR (1, 2), CASTELLANOS N (1), PANETSOS F (1), SANCHEZ-RAMOS C (1)*
*(1) Neuroscience Lab, School of Optics, - UCM, Madrid*
*(2) Lab. Exp. Neurophysiol, H. Ramón y Cajal, Madrid*

**Purpose** The lateral geniculate nucleus (LGN) is the main thalamic relay nucleus for the visual information that arrive from the retina to the cortex. The main issue of the present work is the electrophysiological caracterization of the rat LGN and the response of its neurons to complex visual stimuli.

**Methods** Extracellular multiunit recordings have been performed in Wistar anesthetized rats stimulated with flashes and moving gratings. We have determined the latencies, number of spikes and presence of oscillatory activity in the responses evoked by flashes as well as the spatial distribution of these characteristics among the neurons of the LGN. Complex stimuli represented by moving gratings have allowed us to study the behaviour of the neurons as a function of orientation, velocity and spatial frequency of the stimuli.

**Results** The neuronal response patterns change both linearly and non-linearly depending on these parameters showing in the majority of the cases an increase in their latency with respect of flash stimulation and oscillation frequencies in all ranges except α.

**Conclusion** One key tool for our study has been the variation coeficient of averaged spiking frequency of neuronal population which show us that bars orientation is a very important parameter.

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## Effect of a polarized on photopic contrast sensitivity and disability glare

*DEL MORAL MARTÍNEZ MI, SANCHEZ-RAMOS C, PUELL MC, PEREZ-CARRASCO MJ, LANGA A*
*Departamento de Óptica, Universidad Complutense, Madrid*

**Purpose** To establish the effect of a solar polarized filter on photopic contrast sensitivity (CS) determined with and without glare and on disability glare.

**Methods** Natural sunlight is partly polarized and polarized filters are the only way capable of blocking the glare produced by reflection. Other types of solar filters only reduce the level of luminance.CS with and without glare were determined using the CGT 1000 (6 stimulus sizes –6.3 to 0.7 degrees; 12 levels of contrast –0.01 to 0.45; 8 peripheral glare sources) at photopic luminance level and under different conditions: without filter (to avoid the patient learning effect) or using a graphite or polarized filter on an alternating basis. The study sample was comprised of 82 subjects aged 18 to 95 (56± 21) years.

**Results** Differences in disability glare were recorded for intermediate stimulus sizes between both filters. Differences statistically significants in disability glare according to the filter versus without filter were recorded for intermediate stimulus sizes. CS values for intermediate stimulus determined in the presence of glare differed significantly when comparing the use of no filter versus both filters, or between both filters with glare.

**Conclusion** The use of solar filters improves CS for intermediate stimulus sizes, being highest values using the polarized filter

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## Monochromatic Ocular Aberrations in Professional Tennis Players

*GICQUEL JJ (1), NGUYEN-KHOA JL (2), CHÂTEAU N (3), DIGHIERO PLD (1)*
*(1) CHU Jean Bernard / Ophthalmology, Poitiers*
*(2) Ophthalmologie / Clinique de la Vision, Paris*
*(3) Optics, Orsay*

**Purpose** To report monochromatic aberrations distribution in a population of professional French tennis players with a non corrected monocular visual acuity of 20/20.

**Methods** Inclusion criteria are: professional tennis player, monocular visual acuity superior or equal to 20/20 (determined with a Landolt rings chart). Sphere measurement has to be inferior to 0,5 Diopter and astigmatism less than 0,75 Diopter.A Shack-Hartmann type aberometer is used. 3 measurements are performed: without and with dilation (5% Neosynephrin).

**Results** Compared to the literature data on normal subjects, the global level of ocular aberrations (total RMS) of this specific population is low. There seems to be a correlation between both eyes for spherical aberration, astigmatisms with a mirror symmetry (enantiomorphism) and for aberrations with oblique variation axis (Coma 0, Trefoil O). Spherical aberration is the most common aberration observed in pilots with a natural "Super Vision". It might play a role in compensating retinal image quality.

**Conclusion** In this specific population, higher order aberrations do exist. The good visual performances and sport abilities of these subjects are not correlated with a complete absence of ocular aberrations. Associations of selected ocular aberrations might even be beneficial. Aberrations distribution in this population may help define new standards for specific sport aptitudes.

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## Refractive error changes in young adults during a period of 3 years

*JORGE J (1), QUEIROS A (1), ALMEIDA JB (2), PARAFITA MA (3)*
*(1) Physics (Optometry), Braga*
*(2) Physics, Braga*
*(3) Surgery (Ophthalmology), Santiago de Compostela*

**Purpose** This study was initiated to assess the prevalence and changes of refractive state in university student's population in Portugal during a period of three years

**Methods** In a longitudinal study 118 universitary students (34 males and 84 females) with a mean age 20.5 ± 2.33 years (mean ± SD), received two visual examinations at intervals ranging from 30 to 38 months. The results obtained by the subjective distant refractive with cycloplegia method were used in the analysis and the refractive values were converted into spherical equivalents (SER) for some analysis. Myopia was defined as SER<=-0.50D, emmetropia as SER >-0.50D and <+0.50D and hyperopia as SER >=+0.50 D

**Results** The refractive error of the sample in the 1st visit, ranged from –6.75 to +3.00 D mean spherical equivalent + 0.20 ± 1.53D (Mean ± SD.). The maximum amount of astigmatism was –2.25 D. The incidence of refractive errors in the 1st visit was 22.0% of the students had myopia, 28.8% had emmetropia and 49.2% had hyperopia and for the 2nd visit was 27.1% of the students had myopia, 33.1% had emmetropia and 39.8% had hyperopia.There are statistically significant differences between 1st and 2nd visit (1st mean refractive error (MRE)=0.04 ± 1.49D; 2nd MRE=-0.25 ± 1.72D). The mean diferrence between the two visits was 0.29 ± 0.38 D, p <0.001. We verify that 17.6% of the students with emmetropia became myopic and 19% of the students with hyperopia became emmetropic (p<0.001). We also verify that 72.9% change value of the refractive error in the myopic direction and 39.8% experiment a changes greater than 0.38D in SER

**Conclusion** The results show an incidence of myopia similar to the other results in other countries. The results show a significant and preoccupant myopia shift

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## The impact of long myopic defocus on eye growth and refractogenesis in children with hyperopia

*TARUTTA EP*
*Laboratory of Myopic Studies, Moscow*

**Purpose** To study the effect of permanent lasting overcorrection of hyperopia on eye growth and refraction in children.

**Methods** 18 children (36 eyes) with microesotropia and amblyopia averagely aged 5.8 years with the mean R=+2.77D on the better eye, +3.2D on the worse, BCVA 0.82 and 0.4, axial length (AL) 21.1 mm and 20.9 mm, respectively, were 5 years followed up. The correction lens power of the better seeing eye exceeded the refraction by 1.5-2.5 D in order to reduce VA down to 0.2-0.3, whilst for the worse eye it was equal to refraction. The control group was composed of 15 children of a similar age with the mean R=+3.14D and the mean AL 21.2 mm, who wore regular correction lenses.

**Results** 3 months after start of wearing glasses, a 1.25-2.0 D increase of hyperopia degree was marked in 14 cases on the "penalized" eye, which drew the eye's refraction closer to the power of the penalizing lens, brought about an increase in VA and made the eye predominant. Obviously, the adaptive effect was achieved due to the change of the habitual accommodation tonus and to manifestation of residual hyperopia. After a respective increase of the positive lens power, the effect was not repeated. By the end of follow-up period (average age 10.8) the refraction and AL parameters showed no noticeable change: respectively, 2.73 D and 21.5 mm on the better eye and 3.34 D and 21.3 mm on the worse eye. In the control group, the mean refraction was 1.91 D at the end of the follow-up period and the mean AL was 22.7 mm.

**Conclusion** Full correction and overcorrection of hyperopia delayed age-related refraction increase and AL growth in children who wore penalizing spectacles on a permanent basis.

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## Effect of lasik on pupil size and accommodation in myopic patients

*GIL R (1, 2), HERNANDEZ VERDEJO JL (1, 2), RAMZY M (3), TELIS MA (1, 4), FUENTES I (1)*
*(1) Vissum Hospital Oftalmológico de Madrid, Madrid*
*(2) Universidad Complutense de Madrid, Madrid*
*(3) Vissum Hospital Oftalmológico de Alicante, Alicante*
*(4) Universidad de Alcalá, Alcalá de Henares*

**Purpose** To study the effect of lasik on pupil size and accommodation in myopic patients.

**Methods** This is a prospective single masked study. 53 healthy individuals the photopic and scotopic pupil size and the amplitude of accommodation were measured with a digital (Procyon) and Colvard pupilometer and with the minus lens technique respectively. Evaluation of parasympathetic innervation of pupil was performed using a dilute concentration of pilocarpine 0.125%. The paired T-student test and Wilcoxon signed rank test were applied for statistical analysis when needed.

**Results** We found a statisticaly significant increase of pupil size 1 week after lasik from a 5.9±1,1 m.m to 6,14±0,8 m.m for mesopic conditions and from 6.5±1,1 m.m. to 6,9±0,8 m.m. for scotopic conditions (p=0,009 and p=0,0001 respectively). After instilation of dilute pilocarpine we do not find change of pupil size 1 month after lasik for a group of 17 patients. The amplitude of accommodation for a group of 36 patients remained reduced at 1 month form a preop value of 8.05 ± 2.06 D to, 6.58 ± 1.37D (p = 0.004)

**Conclusion** Lasik seems to induce temporal changes of pupil size and accommodative function during the early postop period. These changes do not seem to be due to denervational reasons.

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## Accommodative response after ICL implantation in myopic eyes

*SHELUDCHENKO NV, AVETISOV SE, SHELUDCHENKO VM*
*State Eye Research Institute of Russian Academy of Science, Moscow*

**Purpose** Implantation of Implantable Collamer Lenses (ICL) in cases of high myopia is the modern surgical procedure. The lens design and implantation technology have changed greatly. But it is a question about influence of ICL to the accommodation of the eye especially during visual work. The aim of research is to investigate the accommodative response with different stimulus in myopic eyes with ICL.

**Methods** We have observed 11 patients (19 eyes) from 20 to 27 years old with myopia from 6 to 14 D. Accommodometer AA2000 Nidek was used for accommodative response measuring. We measured slow accommodative reaction with and without tension faze and fast reaction with stimulus 1, 2 and 3 D 3 month after surgical procedure. ICL STAAR posterior phakic lens were implanted to the all eyes with injector through 3 mm corneal incision.

**Results** After operation visual acuity was 0.87±0.09. After operation we found that the amplitude has increased 2 times. The steady tension of accommodation has decreased. We detected the increasing of the accommodative amplitude with fast reaction to increasing of stimulus (from 0.6 to 0.8; from 1.1 to 1.75; from 1.7 to 2.6 D respectively).

**Conclusion** In all cases ICL of new generation didn't bother the work of accommodation. Moreover, the increasing of power of accommodation response was detected, but its steady decreased a little.

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## A randomised cross-over trial to assess vision-related quality of life with an autofocus bioptic telescope

*HARPER RA (1), CHANDLER C (1), REEVES BC (2), SZNAPKA J (1), DICKINSON CM (3)*
*(1) Manchester Royal Eye Hospital, Manchester*
*(2) Department of Medicine, Bristol University, Bristol*
*(3) Optometry and Neurosciences, Manchester*

**Purpose** To determine the impact of an autofocus bioptic telescope on quality of life (QOL) in subjects with low vision in comparison with existing low vision aids (LVAs).

**Methods** Ninety-six participants (62 males and 34 females, median age 67.5 years) with low vision (VA worse than 0.5 logMAR) were randomised into 1 of 2 groups: Group 1 received the Ocutech VES autofocus telescope in a first 2-month period, and group 2 received the Ocutech in a subsequent 2-month period. When without the Ocutech, participants continued using their existing telescopes/other LVAs. The NEI-VFQ 25 and LVQOL were administered at baseline and at the end of each 2 month period (i.e. after periods with and without the bioptic device).

**Results** Repeated measures ANOVA revealed a significant effect on the NEI-VFQ composite score, the distance vision sub-scale and the LVQOL distance vision sub-scale with the Ocutech in comparison to existing LVAs ($p<0.0001$). Subjects tended to use the bioptic device for more prolonged periods of time than conventional telescopes (67% versus 31% continuous use for >30 minutes) and extended their usage to include tasks that were less feasible with conventional telescopes.

**Conclusion** These findings suggest that an autofocus bioptic telescope can improve vision-related QOL in comparison with existing LVAs. The profile of use of the device also supports these QoL findings, although issues including weight, comfort and device appearance are significant limitations.

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## Variation of visual symptoms in terms of type and degree of ametropia

*VIDAL LÓPEZ J, SOBRADO CALVO P*
*Oftalmología, Optometría, Otorrinolaringología y Anatomía Patológica, Murcia*

**Purpose** The aim of this study was to assess if the type or degree of ametropia are related with visual symptoms associated to reading tasks.

**Methods** One hundred and sixty-five (103 males and 62 females) High Schools students were evaluated using a Visual Symptoms Inventory (Vidal, 2005), retinoscopy and auto-refractor. Currently spheric and toric power of glasses was registered. These data allow us to compute: the spheric and cylindric ametropia in both eyes per patient and the uncorrected ametropia. Patients with anisometropia greater than 1 diopter were excluded. Spheric and cylindric ametropia of both eyes was averaged in all patients. Participants were divided into eight groups: type of ametropia (spheric or cylindric) x degree of ametropia (high or low) x optical correction (corrected or uncorrected ametropia).

**Results** Patients with high spheric ametropias had more visual symptoms than patients with low ametropias both in total estimated spheric ametropia (t=2.06, df=140, p=0.04) as in uncorrected ametropia (t=2.41, df=140, p=0.02). Cylindric ametropias were scarce association with visual symptoms in both conditions: total estimated astigmatism (means contrast in symptoms between groups with high and low astigmatism: t=1.71, df=140, p=0.09) and uncorrected astigmatisms (t=1.64, df=140, p=0.10). Differences in symptoms between groups with high and low uncorrected ametropias were due to the results obtained in patients with hyperopia (t=2.72, df=55, p=0.01). Patiens with corrected or uncorrected myopia did not show statistical differences in symptoms scores (t=0.03, df=76, p=0.97).

**Conclusion** Only uncorrected hyperopes with spheric ametropias greater than +0.50 D have (significantly) more symptoms than corrected hyperopes.

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## Associations between specific measures of vision and Vision-Related Quality of Life in patients with Bothnia Dystrophy, a defined type of Retinitis Pigmentosa

*BURSTEDT MSI, MÖNESTAM E, SANDGREN O*
*Department of Clinical Sciences/ Ophthalmology, University of Umeå, Umeå*

**Purpose** To assess the relationship between objective tests of visual function and vision-related quality of life in patients affected with Bothnia Dystrophy (BD), a retinal dystrophy of retinitis pigmentosa type with progressive maculopathy.

**Methods** Forty-nine patients were tested. Weighted distance logMAR visual acuity (WVA), weighted logMAR low contrast VA (WCS), and binocular visual field (VF) areas were calculated. Vision-Related Quality of life was assessed using the 25-item NEI-Visual Function Questionnaire (VFQ-25). Correlation statistics were used and adjusted analyses of the relationship between the composite score and the objective visual function tests were performed with multiple linear regression.

**Results** VRQL was significantly correlated with age, WVA, WCS, and binocular VF areas ($p<0.001$). Calculation of partial correlation coefficients showed age to be significantly correlated only with VF (V-4-e) area ($p<0.0001$). Multiple linear regression analyzes revealed age and WVA to be significantly associated with the NEI-VFQ-25 composite score ($p<0.02$ and $p=0.001$, respectively). WVA alone was the strongest predictor of self-reported experience of total visual function in BD patients ($r2=0.69$).

**Conclusion** A strong relationship between objective tests of visual function, and patient perceived VRQL as assessed by a questionnaire was found. WVA was the strongest predictor and together with age explained almost 70% of the variability of the composite score of the questionnaire.

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## Tinted contact lenses in Bothnia dystrophy

*JONSSON ÅC, SANDGREN O, BURSTEDT MSI*
*Umea University/Clinical Sciences/Ophthalmology, Umea*

**Purpose** To determine whether dark tinted contact lenses can improve the visual function for patients with Bothnia dystrophy (BD), a variant of ARRP with an extremely delayed dark adaptation.

**Methods** Twelve patients with BD participated in the study. Their visual acuity varied in a range from 0.05 to 1.0 decimal visual acuity. All patients were fit with the same type of soft contact lens tinted dark brown. After wearing the lenses for approximately one month an evaluation was made. Visual acuity, contrast vision, near vision and visual fields were tested, both before the contact lens fitting and at the one-month follow up. At these two occasions the patients also answered a visual-function questionnaire. The physical properties of the lenses were tested using spectrophotometry.

**Results** Nine of the twelve patients have chosen to continue wearing dark contact lenses after the end of the study. Three patients, belonging to the highest visual acuity group, decided to terminate contact lens wear after the initial month of study. The patients with the lowest visual acuity were most grateful about the lenses and describe an immense improvement of their visual function. The optimal colour of the lenses preferred by the patients, differs from person to person and from season to season.

**Conclusion** Dark tinted contact lenses can make a huge improvement in visual function for patients with BD. Individuals with very low visual acuity appears to benefit the most from dark tinted contact lenses compared to those who have better visual acuity. Traditional vision tests like visual acuity test and contrast sensitivity test does not reflect the subjective improvement of the visual quality that many of the participants describe.

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## Electrophysiological Changes of Retinal Ganglion Cells in RCS Rats during Retinal Degeneration

*YIN ZQ (1), CHEN ZS (1), CHEN S (2), WANG SJ (1)*
*(1) Southwest Eye Hospital, Southwest Hospital, Chongqing*
*(2) Dept of Ophthalmology, the Second Hospital of Jiangxi Medical College, Nanchang*

**Purpose** Retinitis pigmentosa is one of the main causes of blindness throughout the world and its pathogenesis remains unclear. In this study, patch-clamp recording was performed from ganglion cells in retinal dystrophic RCS and normal rats during development. We recorded from 314 retinal ganglion cells in the three species of rats, dystrophic RCS rats (RCS-p+), Control RCS rat (RCS-rdy+p+), and Normal rats (Long Evans rats). There were three discharge patterns of action potential, single, transient, and sustained firing in ganglion cells of RCS rats. The main discharge pattern was single firing in P1-2W, then were transient and sustained firing. However, during later stages (after 7-8 weeks) of retinal degeneration, action potential was reduced in amplitude and frequency, and was even lost in RCS rats. These findings differed dramatically from those of rats without retinal degeneration, suggesting there are functional ganglion cells in RCS rats early, but part lose function during retinal degeneration, even though morphological differences are not apparent. Understanding the changes of electrophysiological characteristics during retinal degeneration in detail may help explore the optimal time course for treating retinitis pigmentosa.Supported by the Chinese NSFC (Grant No. 30025014, 30270457).

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## Cross – Infection and Contact Ophthalmic Devices: Clinical Trials of a Disposable Ophthalmic Barrier System

*FRANKLIN V (1), HENLEY C (1), TIGHE B (1), KERR J (2), GEE H (3), FLANAGAN J*
*(1) Biomaterials Research Unit, Birmingham*
*(2) University Hospital, Croydon*
*(3) First water Ltd, Ramsbury*

**Purpose** It is generally accepted that there is a potential risk of cross-infection from patient to patient from contact ophthalmic devices such as the Goldmann tonometer, Gonioscope lenses, A-scan ultra sound probes, and ultrasound pachymeters. Current approaches to address this of risk of cross-infection are either expensive, or inefficient. Therefore an alternative solution needs to be found.A four-layer barrier system prototype has been developed for use in conjunction with the Goldmann tonometer, and has undergone clinical trials.

**Methods** 69 patients were recruited from Glaucoma Clinics at Moorfields Eye Hospital, London, UK. The IOP was measured by slit-lamp mounted applanation tonometry using a disposable TonojetTM tonometer tip. 2 IOP readings were recorded for each eye under each condition i.e. both with and without the use of the barrier system. The order of measurement was randomised and the readings were masked from the clinician carrying out the procedure.

**Results** The results of the clinical trials to determine the validity and reproducibility of intraocular pressure (IOP) measurements with the use of the barrier system prototype will be presented.

**Conclusion** *There was no significant difference in IOP measurement with or without the use of the novel barrier system. Therefore it can be concluded that the barrier system does not effect the validity and reproducibility of IOP readings*There was no evidence of protein contamination when the barrier system was used. Therefore it has effective barrier properties against proteins

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## Adhesion properties of ocular isolates of Staphylococcus epidermidis

*PINNA A (1), SECHI LA (2), ZANETTI S (2), USAI D (2), DESSOLE L (1), CARTA F (1)*
*(1) Institute of Ophthalmology, University of Sassari, Sassari*
*(2) Department of Biomedical Sciences, Section of Experimental and Clinical Microbiology, Sassari*

**Purpose** To report on the adhesion properties of Staphylococcus epidermidis strains isolated from patients with keratitis, purulent conjunctivitis, and chronic blepharitis.

**Methods** Conjunctival swabs and corneal scrapings were inoculated for culture. S. epidermidis identification was performed using biochemical tests. The staphylococcal ability to adhere to human conjunctival epithelial cells (WKD cells) and form biofilm on a abiotic surface was assayed. In addition, polymerase chain reaction (PCR) amplification was used to investigate whether the isolates under study carried virulence genes involved in staphylococcal adherence and biofilm production, such as the ica and embp genes.

**Results** Twenty-three S. epidermidis strains were isolated. Adherence to WKD cells was weak (0-20 bacteria/cell) in 9 cases, medium (21-80 bacteria/cell) in 10 cases, and strong (> 80 bacteria/cell) in 4 cases. All strains showed strong biofilm production (OD595>3). PCR amplification revealed that 17 strains had the embp gene and 3 strains had the ica gene. All the strains with the ica gene also had the embp gene.

**Conclusion** Results suggest that the ability to form biofilm and adhere to conjunctival epithelial cells may play a role in the pathogenesis of S. epidermidis-related external eye infections.

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## Serum Prolactin Levels in HLA B27-Associated Uveitis

*PROENCA H, LOPES D, FERREIRA C, MIRANDA M, CASTANHEIRA-DINIS A, MONTEIRO GRILLO M*
*(1) Ophthalmology, Lisbon*

**Purpose** To evaluate basal serum prolactin levels in patients with HLA-B27 uveitis.

**Methods** We analised 33 patients with HLA-B27 uveitis and 30 heathy controls. 14 out of 23 patients with arthritic disease had ankylosing spondylitis.

**Results** Prolactinemia was significantly higher (mean=15,84 ng/mL) in patients vs. controls (mean=11,50 ng/mL) (p=0,026). Subgroup analysis reveled prolactinemia in arthritic disease patients (mean=17,23 ng/mL) significantly higher than controls (mean=11,50 ng/mL) (p=0.006) and in ankylosing spondylitis (mean=17,20 ng/mL) vs. controls (mean=10,13 ng/mL) (p=0,005). Autoimmunity features also correlated with higher prolactinemia (mean=17,26 ng/mL) vs. controls (mean=8,64 ng/mL) (p=0,004).

**Conclusion** These results suggest the role of serum prolactin levels in HLA B27-associated uveitis pathogenesis and its major subgroups.

■ 472

## Immunofluorescence against immunohistochemistry in the diagnosis of ocular conjunctival immune diseases

*FERNANDEZ ALONSO N (1), MENDEZ MC (1), PIGAZO JM (1), SAORNIL MA (1), HERRERAS JM (2), CALONGE M (2)*
*(1) Registro de Patologia Ocular Miguel N. Burnier. Instituto Universitario de Oftalmobiología Aplicada (IOBA), Valladolid*
*(2) Grupo de superficie ocular. Instituto Universitario de Oftalmobiología Aplicada (IOBA), Valladolid*

**Purpose** The diagnosis of ocular pemphigus or pemphigoid has been made by immunohistochemical (IHQ) methods in our laboratory till 2002. But in the last two years, this diagnosis is made by immunofluorescence methods (IF). The purpose of this paper is to compare both techniques, seeing its advantages and disadvantages, and to justify the use of IF instead of IHQ in the diagnosis of ocular immune diseases.

**Methods** Four conjunctival biopsies were studied using both methods, IHQ and IF, and nine antibodies: IgG, IgA, IgM, IgD, IgE, C3, C4, albumin, collagen type IV (as a positive control), and a negative control.IHQ method: An indirect immunoperoxidase was performed, using an Biotin-Streptavidin Amplified (B-SA) detection system with Diaminobenzidine as crhomogen. IF method: A direct IF was used, using fluorescein marked antibodies, except for collagen type IV, were an indirect IF was performed.

**Results** Similar results were obtained using both techniques: collagen type IV marked epithelial basal membranes, and biopsies diagnosed as pemphigoid showed line drawing basal membrane deposits for similar antibodies, usually IgG, C3 and C4. However, basal membrane deposits are better seen by IF, although this technique is not permanent.

**Conclusion** IF method is faster and more sensitive than IHQ for the diagnosis of ocular immune diseases.*partially funded by grant "Jose Mª Aguilar Bartolomé"

## ▪ 473
## Rhinocerebral Mucormycosis

*PLAZAS A, RUIZ-LAPUENTE CJ, DEL ESTAD A*
*Ophthalmology (HH UU Virgen del Rocío), Sevilla*

**Purpose** To report the incidence and prognosis of patients undergoing Rhinocerebral Mucormycosis in a terciary reference centre in the last two years.

**Methods** Case report: Mucormycosis is a rare, life threatening , opportunistic infection caused by fungi in the class of Phycomycetes. Orbitorhinocerebral mucormycosis, the most common type, generally occurs in patients undergoing Immunosuppression and/or metabolic abnormalities. Pulmonary and disseminated form have been described as well. We report two cases of patients affected by this devastating disease.Case 1: A 17-years-old man, undergoing diabetic ketoacidosis, was diagnosed of necrotic sinusitis developed a central retinal artery occlusion in the left eye and keratouveitis in the right eye. He was diagnosed of rhinocerebral mucormycosis associated with cavernous sinus thrombosis, and underwent medical treatment and surgical debridement of rhinosinual lesions, but he refused any ocular surgery. Case 2: A 72-years-old man affected of orbitorhinocerebral mucormycosis was referred to our centre to perform an orbitary exenteration.

**Results** Case 1: Early medical and surgical treatment led to a good outcome in this case, retaining useful visual acuity in the right eye (follow up: 18 months).Case2:In spite of the agressive surgery and medical treatment, the patient died in ten days.

**Conclusion** Mucormycosis is a rare and acute fungal infection which is frequently lethal. The diagnosis of rhinocerebral mucormycosis should be considered in the clinical setting of necrotic sinusitis and acute neurologic deficit in diabetic patients. However, in selected cases early medical and surgical treatment may led to a good outcome.

## ▪ 474
## Bacterial identification using PCR in acute endophthalmitis

*CHIQUET C (1), LAFONTAINE PO (2), VINH V (1), THURET G (3), BENITO Y (4), CREUZOT-GARCHER C (2), ROUBEROL F (5), PALOMBI K (1), DENIS P (5), BRON AM (2), ROMANET J-P (1), VANDENESCH F (4)*
*(1) Department of Ophthalmology, Grenoble*
*(2) Department of Ophthalmology, Dijon*
*(3) Department of Ophthalmology, Saint-Etienne*
*(4) Department of Microbiology, Lyon*
*(5) Department of Ophthalmology, Lyon*

**Purpose** To improve identification and speciation of bacteria involved in acute endophthalmitis, using polymerase chain reaction (PCR) technique

**Methods** This prospective study includes 48 patients with endophthalmitis after cataract surgery (n = 37), strabismus surgery (n = 1), vitreo-retinal surgery (n=1), combined surgery (n=1, cataract and epiretinal membrane) or trauma (n=5). In emergency, an anterior chamber puncture (n= 32) and/or a vitreous puncture (n=18) were performed. Aqueous humor and/or vitreous samples are collected: 100 microL for standard culture (Brain Heart Infusion) and 150 microL for PCR.

**Results** After aqueous humor puncture (n=32), microbiological diagnosis is performed on 31% of the samples using cultures and on 38 % using PCR. The association of PCR and cultures allows the identification of the bacteria in 40 % of the cases. After vitreous puncture (n=18), cultures were positive in 56% and PCR was positive in 69%. For aqueous or vitreous samples, the correlation between both techniques is 100%. After vitrectomy (n = 23), the microbiological diagnosis was made in 15% using cultures and in 77% using PCR. Considering aqueous humor and vitreous samples (puncture and vitrectomy), the infectious agent was identified in 75% of the cases.

**Conclusion** These results confirm that the association of culture and PCR improves the identification of the causative pathogens in endophthalmitis. These preliminary results suggest that cultures and PCR performed on vitreous samples allow a higher rate of bacterial identification than on aqueous humor.

## ▪ 475
## Intravitreal Moxifloxacin monotherapy of endophthalmitis in an experimental model

*MONTERO-MORENO JA (1), FERRER C (1), RODRIGUEZ AE (1), FERNANDEZ J (1), ABAD JL (1), ALIO JL (1, 2)*
*(1) Vissum - Instituto Oftalmológico de Alicante, Alicante*
*(2) Dpto. de Patología y Cirugía, Universidad Miguel Hernández, Alicante*

**Purpose** To evaluate the efficacy of intravitreal moxifloxacin 0.2% monotherapy versus combined therapy in an experimental model of endophthalmitis.

**Methods** 75 New Zealand rabbits received injections of either Staphylococcus aureus (40 CFU/0,1 ml), Staphylococcus epidermidis (40.000 CFU/0,1ml) or Pseudomonas aeruginosa (40 UFC/01 ml) into the vitreous cavity of the right eye. Three subgroups were formed; treatment with 100 µl moxifloxacin 0.2% (10 rabbits), treatment with 100 µl of vancomycin 1% and 100 µl of ceftazidime 2% (10 rabbits), and an untreated control subgroup (5 rabbits). The Peyman classification was used to assess the severity of endophthalmitis. Eye examinations were performed until day 8 from inoculation. The microbiological status of vitreous and aqueous humor samples was tested at day 2, 3, 5 and 8.

**Results** In the Pseudomonas group, 90% of the rabbits treated with moxifloxacin did not develop severe endophthalmitis, versus 50% of the rabbits treated with the combined therapy, however microbiological results showed 80% versus 100%, respectively. In the S. aureus group, 100% of the rabbits treated with moxifloxacin did not develop severe endophthalmitis, versus 70% of the rabbits treated with the combined therapy, microbiological results showed 100% versus 70%, respectively. In the last group (S. epidermidis) both treatments (combined and monotherapy) showed the same result, 100% did not develop severe endophthalmitis and 100% of the cultures were negative.

**Conclusion** This dose of moxifloxacin (0.2%) was considered as or more effective in treating endophthalmitis caused by Staphylococci and Pseudomonas than a combination of vancomycin and ceftazidime.

## ▪ 476 / 4263
## Serum Prolactin Levels and Behçet Disease

*PROENCA H, GONÇALVES C, FERREIRA C, MIRANDA M, CASTANHEIRA-DINIS A, MONTEIRO GRILLO M*
*Ophthalmology, Lisbon*

**Purpose** To evaluate basal prolactinemia in Behçet's Disease (BD) and correlate with phenotypic expression.

**Methods** We compared 22 patients fulfilling BD Research Committee criteria with 21 age- and sex-matched healthy control subjects. Age, clinical manifestations, HLA-phenotyping and therapy were analysed.

**Results** Prolactinemia was significantly higher (mean=19,34 ng/mL) in BD's patients vs. controls (mean=9,83 ng/mL) (p=0,039). This difference was even higher in complete-type BD subgroup vs. controls (p=0,001). Younger patients (<30 y) required corticosteroids plus immunosuppressives more often (80%) suggesting a correlation between age and disease severity.

**Conclusion** Results suggest the role of prolactin in BD pathogenesis and its correlation with disease expression, especially in complete-type BD.

■ **477 / 4267**

## Contribution of lumbar puncture and CSF analysis in the diagnosis of Vogt-Koyanagi-Harada disease

*BOUCHENAKI N (1, 2), HERBORT CP (2, 3, 4)*
*(1) Memorial A. de Rothschild, Clinique Générale-Beaulieu, Geneva*
*(2) Inflammatory Eye Diseases, La Source Eye Centre, Lausanne*
*(3) Memorial A. de Rotschild, Clinique Générale-Beaulieu, Geneva*
*(4) Lausanne University, Lausanne*

**Purpose** Vogt-Koyanagi-Harada disease (VKH) is characterized by a bilateral granulomatous uveitis with exudative retinal detachments associated with systemic neurological, auditory, tegumentary manifestations. VKH is relatively unfrequent in Europe, therefore diagnosis is often reached with some delay. The aim was to determine the utility of lumbar puncture (CSF analysis) in the diagnosis of VKH disease in a non endemic setting.

**Methods** The charts of patients with the diagnosis of VKH seen at la Source Eye Centre in Lausanne from 1.1995 to 1.2005 were analysed. The delay from onset of the disease to diagnosis was established. Different diagnostic investigations were analysed and, in particular, their contribution toward diagnosis was evaluated. The proportion of patients were CSF analysis confirmed neurological involvement or, represented the only parameter indicating neurological involvement was analysed.

**Results** Twenty patients with VKH disease were seen in our centre. 9 patients (45%) presented neurological symptoms (headache, meningismus...) before ocular manifestations. 14 patients (70%) underwent lumbar puncture with CSF analysis and 12 (86%) presented a lymphopleocytosis. The 2 negative CSFs came from patients in the chronic stage and under treatment. In 5/12 patients (45%) CSF pleocytosis was the only neurological sign. In 3 patients, the association of CSF pleocytosis and the presence of choroidal lesions on the indocyanine green angiography (ICGA) allowed the diagnosis of VKH disease.

**Conclusion** CSF analysis can contribute to the diagnosis of VKH disease in non endemic areas, even in the absence of neurological symptoms. The association of CSF pleocytosis and the presence of choroidal signs on ICGA can help to confirm VKH diagnosis.

■ **478 / 4365**

## Complications of Pars Plana Vitrectomy in Acute Endophthalmitis

*GARCHER CREUZOT CP (1), LAFONTAINE PO (1), PASSEMARD M (1), ROUBEROL F (1), CORNUT PL (2), BRON AM (1), THURET G (3), ROMANET JP (4), CHIQUET C (4)*
*(1) Department of Ophthalmology, Dijon*
*(2) Department of Ophthalmology, Lyon*
*(3) Department of Ophthalmology, Saint-Etienne*
*(4) Department of Ophthalmology, Grenoble*

**Purpose** To assess the results and the complications of vitrectomies performed in acute endophthalmitis.

**Methods** Forty–eight patients aged from 22 to 96 years old (69± 16) were included in this multicenter prospective study. All patients presenting an acute postoperative endophthalmitis and undergoing a vitrectomy as part of their treatment were enrolled.

**Results** Visual acuities were light perception (50%), hand motion (35.5%) or between 20/400 to 20/200 (14.5%) before vitrectomy. Vitrectomy was performed with a mean delay of 5.2 days after the onset of the hospitalization. Per operative complications were uncommon: vitreous IOL luxation (n=1), intra vitreous hemorrhages (n=2). Postoperative complications were phthisis (6%), retinal detachment (10.5%), irido–capsular synechiae (12.5%), epiretinal membrane (8%) and ocular hypertension (14.5%). Final visual acuities were 20/20 (8,5%), from 20/40 to 20/25 (21%), from 20/200 to 20/50 (31.5%), count fingers (16.5%), hand motion (6%), light perception (6%) and no light perception (10.5%).

**Conclusion** Vitrectomy performed as a treatment modality for endophthalmitis remains a difficult vitreous surgery due to the poor visibility of the posterior segment (cornea edema, anterior segment's flare, vitreous opacities). The most frequent complications are similar to vitreous surgeries done in other indications than endophthalmitis i.e. retinal detachment, epiretinal membrane and ocular hypertension. Final visual impairment or blindness is mainly due to phthisis or untractable infection rather than vitrectomy.

■ **479 / 4366**

## Comparative study of acute post-operative endophthalmitis with or without microbiological identification

*VINH V (1), CORNUT PL (2), BENITO Y (3), LAFONTAINE PO (4), THURET G (5), ROUBEROL F (2), BRON AM (4), DENIS P (2), CREUZOT-GARCHER C (4), VANDENESCH F (3), ROMANET JP (1), CHIQUET C (1)*
*(1) Ophthalmology, Grenoble*
*(2) Ophthalmology, Lyon*
*(3) Microbiology, Lyon*
*(4) Ophthalmology, Dijon*
*(5) Ophthalmology, Saint-Etienne*

**Purpose** To compare epidemiological and clinical data of patients with acute post operative endophthalmitis with microbiological positive ocular samples and those with negative ocular samples.

**Methods** This multicenter prospective study includes 87 patients hospitalized for a post operative endophthalmitis between 2002 and 2005, as a complication of cataract surgery (n = 77), filtering surgery (n = 6), vitreoretinal surgery (n = 2), strabismus surgery (n = 1) or a radial keratotomy (n = 1). A bacteria was identified in aqueous humour and/or in vitreous, by conventional cultures and polymerase chain reaction (PCR) for 69% patients.

**Results** Microbiological proven endophthalmitis differs from sterile endophtalmitis (reduced delay of onset, higher intraocular pressure, rate of vitrectomy and initial and final visual acuity). Patients with a microbiological proven endophthalmitis were not different from patients with negative cultures and PCR for the following criteria : age of patient, type of the initial surgery, functional (pain, visual loss, redness, photophobia, secretions) and clinical signs (corneal edema, hypopion, hyalitis).

**Conclusion** Using cultures and PCR on ocular samples, the positivity of the microbiological identification is associated with some clinical signs. These findings are partly consistent with previous results of the Endophthalmitis Study group (association of no growth with time from surgery to symptoms, hypopion, initial and final visual acuity, media clarity). However the patient's initial examination is not highly predictive of the results of cultures and PCR.

# COURSES

## EVER 2005

October 5-8, 2005
Vilamoura, Portugal

■ 1321
## Basics of testing and ISCEV Standards

*HOLDER GE*
*Moorfields Eye Hospital, London*

**Purpose** To introduce the basics of clincial electroretinography testing (ERG).

**Methods** The minimum standards recommended by the International Society for Clinical Electrophysiology of Vision (ISCEV) for performing ERG will be described, as will additional techniques such as pattern electroretinography (PERG), ON- and OFF-response recording and S-cone ERG recording.

**Results** The nature of the results in normals will be described, together with the fundamentals of ERG diagnosis.

**Conclusion** Standardised techniques are essential for accurate diagnosis and inter-laboratory comparison

■ 1322
## The application of electrophysiology to a genetic eye disease practice

*LEROY BP (1, 2)*
*(1) Department of Ophthalmology, Ghent University Hospital, Ghent*
*(2) Center for Medical Genetics, Ghent University Hospital, Ghent*

**Purpose** To describe the value of electrophysiological test in a genetic eye disease practice.

**Methods** A case presentation format will be used to illustrate different genetically determined conditions leading to night blindness. Both clinical and electrophysiological phenotypes as well as genotypes will be discussed.

**Results** Phenotypes and genotypes of genetically determined diseases leading to night blindness are very different. An important distinction to be made is the one between stationary and progressive diseases. Indeed, other than for night blindness, the visual outcome may differ considerably between different conditions.

**Conclusion** Genetically determined night blindness is diverse. Visual electrophysiology allows an important distinction between progressive and stationary conditions.

■ 1323
## Multifocal ERGs

*BARBER C*
*Medical Physics, Queen's Medical Centre, Nottingham*

**Purpose** To introduce the multifocal ERG technique and its clinical usefulness.

**Methods** To explain (non-mathematically) the principles of the technique and the display of findings. To illustrate the clinical application by means of case studies.

**Conclusion** This new technique opens many new possibilities in clinical electrophysiology of vision, and it can also be extended to corical signals (multifocal VEPs).

■ 1324
## Diagnostic electrophysiology in inherited disease

*HOLDER GE*
*Moorfields Eye Hospital, London*

**Purpose** To describe the electrophysiological investigation of inherited retinal disease.

**Methods** ISCEV Standard electroretinography (ERG: The International Society for Clinical Electrophysiology of Vision), supplemented by pattern electroretinography (PERG); ON- and OFF- rseponse recording; S-cone ERG recording; and other supplementary ERG techniques.

**Results** The diagnostic features of various inherited retinal diseases will be described, including retinitis pigmentosa, cone and cone-rod dystrophies, congenital stationary night blindness and others.

**Conclusion** Electrophysiological assessment plays a major role in the diagnosis and management of patients with inherited retinal disease.

## ▪ 1341
## Principles and practice of fluorescein angiography

*ABU EL ASRAR AM*
*Department of Ophthalmology, College of Medicine, King Saud University, Riyadh*

**Purpose** Fluorescein angiography (FA) uses sodium fluorescein, which has its maximal fluorescent activation between 485 and 500 nm, with maximal emission between 520 and 530 nm. FA is especially useful in patients with posterior uveitis to demonstrate the presence of specific changes secondary to intraocular inflammation, such as leakage of dye from the retinal capillaries in the macula (cystoid macular edema) and in the optic disc (optic disc edema). FA studies allow the recognition of retinal vascular inflammation and occlusion resulting from vasculitis in a number of uveitic syndromes. Angiography is useful in recognizing leakage at level of the retinal pigment epithelium in a number of uveitic disorders, including Vogt-Koyanagi-Harada disease and sympathetic ophthalmia. FA is useful in patients with active retinochoroiditis as it demonstrates early hypofluorescence followed by late staining. Angiography is also useful in recognizing retinal and choroidal neovascularization in patients with uveitis.

## ▪ 1342
## Principles and practice of indocyanine green angiography (ICGA)

*HERBORT CP*

**Purpose** Among the novel imaging methods that have become available, indocyanine green angiography (ICGA) certainly is the one of the most important acquisitions. ICGA allows the imaging access to the choroidal compartment that was not accessible beforehand or only with methods such as ultrasonography giving very gross information on the choroid.The principles of ICGA, that differ fundamentally from fluorescein angiography will be exposed and numerous practical examples will be shown in order to illustrate the use of ICGA.

## ▪ 1343
## Ultrasound biomicroscopy (UBM) in inflammatory diseases

*HERBORT CP (1, 2), TRAN VT (1)*
*(1) Inflammatory Eye Diseases, La Source Eye Centre, Lausanne*
*(2) University of Lausanne, Lausanne*

**Purpose** The utility of high frequency ultrasonography (ultrasound biomicroscopy, UBM) in inflammatory diseases will be exposed based on a study examining the yield of essential information given by UBM

**Methods** Images were acquired with a Humphriies UBM instrument using a 100 megahertz probe as well as with the OTI versatile ultrasound instrument using a 35 megahertz probe.

**Results** Hundred eleven eyes from 77 patients were analysed. UBM findings contributed essential information that allowed a diagnosis to be reached or that influenced treatment in 43% of cases. It yielded positive findings in 91% of cases, enabling assessement of porphological changes in the the iris, ciliary body, retroiridal space and vitreous induced by inflammatory disorders or pseudo-inflammatory disorders.

**Conclusion** For uveitis patients with an inflammatory process situated in the iris, ciliary body, pars plana and retroiridal space, UBM was of great clinical value and improved the management in a significant manner in a biased group of patients

## ▪ 1344
## Ocular coherence tomography (OCT) in inflammatoty diseases

*DE SMET MD (1), VAN VELTHOVEN MEJ (1), VERBRAAK FD (1), ROSEN R (2)*
*(1) Ophthalmology, University of Amsterdam, Amsterdam*
*(2) Ophthalmology, New York University, New York*

**Purpose** Demonstrate the use of optical coherence tomography in the diagnosis and management of patients with ocular disease.

**Methods** The Zeiss Stratus OCT and the OCT Ophthalmoscope from OTI were used in the assessment of patients with a variety of ocular conditions. Quality of OCT images were assessed to determine the impact of media opacity (cataract, vitreous, retina), or the impact of choices made in hardware and software. An attempt was made to identify specific characteristics in a number of conditions (macular hole, puckers, surbretinal membranes, central serous retinopathy, uveitis). Correlation with other imaging techniques was made wherever possible.

**Results** Media opacity variably affects the OCT signal. Cataract has a limited effect (unless subcapsular and dense), while dense vitreous opacities can cast a dark shadow on the retina. While the prototype OCT Ophthalmoscope is limited by its scanning speed, interpolation as performed by the stratus OCT can also be limiting particularly if the scan is of poor quality. Characteristic structural changes are seen in a number of clinical entities. Its presence or resolution does not clearly correlate with changes in function. Correlation with other imaging modalities is often beneficial in interpreting OCT findings.

**Conclusion** OCT is a valuable tool in the assessment of patients with ocular disease and in determining the severity of complications or response to treatment.

■ **1345**

## Uveitic macular edema: quantitative assessment by OCT and correlation with visual acuity

*ABU EL ASRAR AM, AL TURKI RH, AL KARASHI AS, AL KURAYA HS*
*(1) Dept. of Ophthalmology, College of Medicine, King Saud University, Riyadh*

**Purpose** To evaluate the correlation between improvement in visual acuity (VA) and the reduction in central macular thickness (CMT) after treatment in uveitic macular edema.

**Methods** Twenty-nine eyes of 19 consecutive patients were studied prospectively. Inclusion criteria were: (1) verification of macular edema by optical coherence tomography (OCT), (2) adequate media clarity for fundus visualization, and (3) absence of coexisting ocular disease limiting visual potential. All patients had the following ophthalmic examination: best-corrected Snellen VA, slit-lamp examination, indirect ophthalmoscopy, fundus biomicroscopy, and OCT.

**Results** There were 11 males and 8 females with a mean age of 40.58 ± 9.5 years (range, 22 to 54 years). Eleven patients had presumed intraocular tuberculosis, four had Behçet's disease, three had pars planitis, and one had ankylosing spondylitis. At baseline, the mean logarithm of the minimum angle of resolution (logMAR) VA was 0.6841 ± 0.5168 (Snellen equivalent, 20/100), and the mean CMT was 419.5 ± 105.9 µm. All patients received adequate immunosuppressive treatment and patients with pressured intraocular tuberculosis received in addition antituberculous therapy. After a mean follow-up period of 4.3 ± 2.8 months, the mean logMAR VA was 0.231 ± 0.178 (Snellen equivalent, 20/30) and the mean CMT was 250.9 ± 41.9 µm. The improvement in VA and the reduction of CMT were significant ($p<0.0001$ for both comparisons).

**Conclusion** OCT is useful in monitoring the efficacy of treatment in patients with uveitic macular edema. Decreased CMT is significantly associated with improvement in VA.

■ **1346**

## Radiology and Ultrasound in uveitis

*BODAGHI B*
*Ophthalmology, Paris*

**Purpose** An associated condition may be determined in nearly 60-70% of patients with uveitis. Radiology and ultrasound are highly valuable tests in patients with a suspicion of HLA-B27 uveitis, tuberculosis, sarcoidosis, multiple sclerosis, Lyme disease and Wegener's granulomatosis. Interesting results and diagnostic orientation may be obtained in 10% of cases but final diagnosis is based on other criteria such as clinical features and biological findings. Although different techniques such as chest x-ray, CT scan and MRI of head, gallium scan and sacroiliac x-ray provide valuable informations for diagnostic evaluation and help further biopsy procedures, others like sinus x-rays are frequently ordered without any help when used on a routine basis.One of the major interests of these imaging techniques is to exclude a masquerade syndrome, especially primary intraocular lymphoma with CNS involvement. Conventional biomicroscopy is interesting for the evaluation of posterior segment complications such as vitreoretinal proliferation and retinal detachment, particularly in patients with intermediate uveitis of various origins. More recently, high-frequency ultrasound biomicroscopy (UBM) was proposed to assess the inflammatory lesions of the iris, ciliary body, pars plana and peripheral vitreous. Specific UBM signs, present in all patients, may be identified in Toxocara uveitis. The groups of patients that benefited most from UBM examination are those with hypotony and opaque media. UBM should be proposed in all patients with hypotony before cataract surgery in eyes with uveitis. Analysis of the ciliary body may show complete atrophy of ciliary processes with a poor final visual prognosis.

■ **1347**

## The role of imaging reading centers in clinical studies

*PETO T, LEUNG I, HARRIS N, KHATUN M, MOLDON I*
*Moorfields Eye Hospital (MEH), London*

**Purpose** Reading Centres (RC) have been in existance for over 40 years; in the past few years their role in ophthalmic research and practice became an essential one. Recent genetic studies highlighted the importance of prior phenotyping before expensive molecular biology experiments carried out. Clinical trials require objective analysis of ophthalmic photographs to validate study results.

**Methods** In a clinical setting, the detailed analysis of photographs obtained in the community allows for non-ophthalmologist led patient care. It also allows high quality service delivery by providing training, quality control and clinician input for patients attending services such as diabetic retinopathy or glaucoma screening. In case of expensive treatments such as Photodynamic therapy, RCs provide feedback to government services on appropriate treatment usage. Remote access facilities via telemedicine allow clinicians in developing countries around the world to gain access to specialist opinion.

**Results** The RC at MEH is one of the few RCs in the world that provides all of the above-mentioned services to the ophthalmic community. During the last 4 years the RC worked with many countries from South Africa to the Inuit population of Denmark. The results of these studies provided those communities with epidemiological data required for service provision planning. Results from clinical trials, such as the trabeculectomy trial, are leading to changes in post-operative care. Community screening in diabetic retinopathy (DR) and providing expert opinion to sites with telemedicine access led to better DR care for patients in those communities.

**Conclusion** RCs are time-consuming to operate but they provide valuable information for communities, clinicians and policy makers.

# ORAL PRESENTATIONS

EVER 2005

October 5-8, 2005
Vilamoura, Portugal

■ 2111

## Four families with recurrent erosions as the main symptom but with four different phenotypes

*FAGERHOLM PPP, HAMMAR B, DELLBY A*
*Department of Ophthalmology,University Hospital, Linköping*

**Purpose** To describe four large families from different parts of Sweden with corneal erosions as a common symptom. The phenotype are different in all families. Genetic work-up in order to localize the genes and the gene products should provide an opportunity to better understand mechanisms of recurrent erosions in general.

**Methods** The type of heredity was found by establishing a pedigree. In two of the families, previously known loci for autosomal dominant corneal dystrophies have been ruledd out. The phenotype of the diseases have been explored in interviews and by clinical examination.

**Results** All families show an autosomal dominant inheritance. In family I, the erosive symptoms start at the age of 6-12 months. In the other 3 families the debut is between 4-6 years of age. Erosive symptoms decline in all families between 20-40 years. In family I, the cornea develop central kelloids that necessitate corneal grafting in about 50 %. In family II , subepithelial fibrosis in the midperiphery dominate. Late discreete kelloid-like noduli may appear . In family III, the changes develop between 40-50 years of age consist of thin but dense,curved superficiala lines of white fibrosis, with clear cornea in between. In family IV, there are no corneal changes. In family I, a majority of the affected members are cured or much improved by high doses of vitamin B. In family IV , permanent cure is obtained with phototherapeutic keratectomy (PTK). In family II and III, PTK can improve vision.

**Conclusion** We believe that the four families constitute different diseases and that none is a previously genetically mapped dystrophy. The discrepansies found between the families suggest that corneal erosions can origin in several mechanisms.

■ 2112 / 362

## Homozygote cytokeratin 12 mutation in a Danish family with Meesmann's dystrophy

*NIELSEN KN (1), ORNTOFT TFO (2), HJORTDAL JH (1), EHLERS NE (1)*
*(1) Department of Ophthalmology, Aarhus*
*(2) Molecular Diagnostic Laboratory, Aarhus*

**Purpose** Meesmann's dystrophy was first described by Meesmann & Wilke (1939) as myriads of microcysts in the corneal epithelium seen by slit-lamp microscopy. Mutations in the cytokeratin 3 or cytokeratin 12 genes are considered to be responsible for the dystrophy (Nishida 1997, Irvine 1997). We here describe such a mutation in a small Danish family of a mother and her daughter.

**Methods** Clinically the mother showed microcysts in the entire epithelium. By contrast, the daughter presented only discrete cystic changes. Blood samples were collected and DNA was isolated. All 8 exons were sequenced on the 3100 Genetic Analyzer. Primers for exon 1a were: 5'-ccatcttgcagcctatataagtttagc-3' and 5'-cgagagaatacctagagagccacc-3'. DNA sequence analysis was performed with the Sequencher software.

**Results** DNA sequencing of the 1.9 kb coding cytokeratin 12 gene showed a 67C→T mutation in the family. The missense mutation gives rise to amino acid substitution Pro15Ser. The mother was homozygotic 67C→T and the daughter was heterozygotic 67C→T. Both parents to the mother were deceased and the origin to the homozygotic mutation could not be established.

**Conclusion** This mutation in the 5'-end of the gene contributes to the characterization of cytokeratin 12 gene in relation to the dystrophy. Most mutations are identified in a hotspot region (position 410-451) but this new mutation causes an amino acid substitution at the N-terminal of this structural protein.Meesmann's dystrophy is described as an autosomal dominant disorder and so a single mutation in one of the alleles is adequate to cause the disease. These data represent a patient who is homozygote of this rare mutation and who shows more severe changes than the heterozygotic daughter.

■ 2113

## Single Nucleotide Polymorphisms in Vascular Endothelial Growth Factor gene and their Association in patients with Age-Related Macular Degeneration

*CARTER JG (1), LOVELL HC (1), RAMSDEN C (1), TURNER SJ (1), YEUNG A (2), ESCARDO J (1), CHURCHILL AJ (1)*
*(1) Ophthalmology, University of Bristol, Bristol*
*(2) Ophthalmology, Bristol*

**Purpose** To determine whether there is an association between single nucleotide polymorphisms (SNPs) in the vascular endothelial growth factor (VEGF) gene and exudative age-related macular degeneration (AMD).

**Methods** This is a case-control study of 53 patients with AMD and 104 controls. Genotypes for 5 SNPs in introns of VEGF gene (+674C→T, +4618C→T, +5092A→C, +9162C→T & +9512C→T) were determined by allele-specific PCR and a subset confirmed by sequencing. Predicted haplotypes were determined by PHASE v2.1. Resultant genotype and extended haplotype data were analysed by Pearson's $\chi 2$, adjusted for the Bonferroni correction where appropriate.

**Results** We found a statistically significant association between the +674CC genotype and AMD (OR=2.2, 95%CI [1.1, 4.6], p=0.033), and a non-significant association of +5092AA genotype with the controls (p=0.075). Analysis of extended haplotypes produced several potential 'at risk' haplotypes associated with AMD (e.g. TCATT; OR=3.0 [1.3, 7.0] p=0.008), and potential 'protective' haplotypes associated with the controls (e.g. TCACC; OR=0.10 [0.02, 0.40] p=0.0001).

**Conclusion** We have found an association between the +674CC genotype and AMD and several extended haplotypes that appear to predispose to AMD. We have also found extended haplotypes that may be considered 'protective' against AMD. It is known that SNPs can regulate gene transcription and that these control elements commonly occur in introns. It is possible therefore that 'at risk' genotype/extended haplotypes predispose to a more angiogenic ocular environment by affecting the balance between angiogenic and non-angiogenic growth factors.

■ 2114

## Awareness of Adult Refsum's Disease as a cause of retinitis pigmentosa: a survey of UK ophthalmologists

*SHAMS PN (1), DAVIES NP (1), GIBBERD FB (2), WIERZBICKI AS (2), FEHER MD (2)*
*(1) Ophthalmology. Chelsea and Westminster Hospital, London*
*(2) Chelsea and Westminster Hospital, London*

**Purpose** Adult Refsum's Disease is a rare autosomal recessive disorder characterized by retinitis pigmentosa (RP) with usually cerebellar ataxia, polyneuropathy, anosmia, hearing loss and bony abnormalities and is associated with high serum phytanic acid. Several of the clinical features are ameliorated by restriction of dietary phytanic acid intake. The aim of this study was to assess awareness of Adult Refsum's Disease as a cause of RP.

**Methods** A questionnaire was sent to all UK ophthalmology departments listed in the Directory of Training in Ophthalmology from the Royal College of Ophthalmologists

**Results** There was a response rate of 91% from the 159 departments surveyed. The average number of consultants per unit was 5 with a mean prevalence of 10 patients with retinitis pigmentosa seen per annum. One third (33%) of the units had previously seen a case of Adult Refsum's Disease. 50% of respondents said they would measure serum phytanic acid in selected patients with retinitis pigmentosa, but only 4.3% would perform this test in all patients with RP.

**Conclusion** This survey (with its exceptional response rate) has shown that although one third of UK ophthalmologists have previously seen a case of Adult Refsum's Disease, only a small percentage will measure a serum phytanic acid in all patients with RP. Often the retinopathy associated with Adult Refsum's predates the other manifestations and early treatment is beneficial in reducing the sequelae of the disease. We therefore recommend that all patients with RP should have serum phytanic acid measured.

■ **2115 / 363**

## Study of Primary Mutation and Clinical Features of Leber's Hereditary Optic Neuropathy in Chinese Patients

*GUO X, WANG Y, JIA X*
*Molecular Genetics, Zhonshan Ophthalmic Centre, Guangzhou*

**Purpose** To study primary mutation and clinical features in Chinese patients with Leber's hereditary optic neuropathy (LHON).

**Methods** Three primary mtDNA mutations (3460A,11778A and 14484C) of 156 Chinese LHON patients were detected by MSP-PCR, HA-SSCP, RFLP and DNA sequence. The clinical features were analyzed by retrospective study.

**Results** Of the 110 probands, the 11778A mutation was found in 100 probands (90.9%), the 3460A in 2 (1.8%), and the 14484C in 8 (7.3%).16 of 250 eyes with the 11778A mutation recovered a mean final visual acuity of 0.03, whereas 28 of 56 eyes with the 14484C recovered a mean final visual acuity of 0.8.

**Conclusion** In Chinese LHON patients, the 11778A mutation are common. The visual acuity of visual recovery with 14484C were better than one with 11778A mutation.

■ **2116**

## A family with autosomal dominant optic atrophy and cataract (ADOAC) and an electronegative ERG

*VOTRUBA M (1, 2), DAVIES VJ (1), THISELTON D (3), HOLDER GE (4), MOORE AT (4, 5)*
*(1) School of Optometry & Vision Sciences, Cardiff University, Cardiff*
*(2) Cardiff Eye Unit, University Hospital Wales, Cardiff*
*(3) Virginia Institute of Psychiatric & Behavioural Genetics, Richmond, VA*
*(4) Moorfields Eye Hospital, London*
*(5) Institute of Ophthalmology, London*

**Purpose** The gene OPA3 (chromosome 19q13.2-q13.3) was first associated with type III 3-methylglutaconic aciduria (MGA): Costeff optic atrophy syndrome. This is a recessive neuro-ophthalmological syndrome, consisting of early-onset bilateral optic atrophy and later-onset extrapyramidal dysfunction and cognitive deficit. Recently, two heterozygous missense mutations in OPA3 have been reported in patients with dominant optic atrophy and cataract (ADOAC). Thus, different mutations in OPA3 are responsible for cataract and neurological defects and implicate different inheritance patterns. This finding prompted us to screen a panel of patients with optic atrophy in whom mutations in the gene for autosomal dominant optic atrophy (ADOA) (OPA1), mapping to chromosome 3q28-qter, had not been found in a previous survey of mutations.

**Methods** A genetic screen of OPA3, using bi-directional genomic sequencing, was undertaken in a panel of 43 unrelated patients with optic atrophy without mutations in OPA1 . The patients in this cohort comprised AD, AR and sporadic OA and two patients had childhood onset cataract.

**Results** Only the two patients with a history of childhood cataract and optic atrophy were found to have a heterozygous mutation 313C>G (Q105E) in OPA3. One of the patients had a documented blue-dot type cataract by age 2 years and the other had cataract surgery at the age of 4 years. Best corrected visual acuity was 6/60 in one patient and 3/60 in the other. An electronegative ERG was recorded in both individuals.

**Conclusion** ADOAC, an unusual ophthalmic combination of optic atrophy and cataract, can lead to RGC loss and other retinal changes which may result in an electronegative ERG.

■ **2117**

## Characterisation of a new mouse model for retinoschisis

*DALKE C (1), JABLONSKI M (2), FAVOR J (3), PRETSCH W (3), GRAW J (1)*
*(1) Institute of Developmental Genetics, Neuherberg*
*(2) Department of Ophthalmology, Hamilton Eye Institute, Memphis*
*(3) Institute of Human Genetics, Neuherberg*

**Purpose** To analyse the genetic basis of a mouse model (44TNJ) with an aberrant retinal phenotype a linkage analysis and molecular characterization were performed. The mice were received from an ENU-based mutagenesis screen of the TMGC, USA.

**Methods** The retinal function of the mice was tested by dark-adapted ERG. Histological examinations of the eyes were performed according to standard procedures. To locate the mutated gene a genome-wide mapping was done and candidate genes were sequenced.

**Results** The 44TNJ mutant mice are characterised by a reduced ERG responses of both a- and b-waves. Histology shows a disruption of the lamination of the retina, particularly of the outer nuclear layer, and holes in the inner nuclear layer. Mapping analysis revealed linkage to the X-chromosome closest to marker DXMit117. Considering the calculated recombination frequency, the retinoschisis-1 homologue gene (Rs1h) was a good candidate. The sequence analysis of the Rs1h gene revealed a point mutation (T to C) of the second base in intron 2. This T to C exchange creates a new restriction site for NlaIII, which is not present in the wild-type gene. The presence of this novel restriction site was confirmed in 5 male mice of the mutant line, however, this restriction site was not found in wild-type controls of C57BL6, C3H, BALB/c, 129 and JF1 mice. In a PCR amplification of the Rs1h cDNA from 44TNJ mutants two products were received, instead of one from wild-type controls. The sequence analysis showed that one product from mutants contains a 10 bp insertion, identical to the first bases of intron 2, except a T to C change of the second base. Sequencing of the other product revealed a 26 bp deletion of the 3´ end of exon 2.

**Conclusion** The 44TNJ mice are a new model for retinoschisis.

## 2122
## Organ culture

*PELS E, MAAS H*
*Cornea Bank Netherlands Ophthalmic Research Institute, Amsterdam*

**Purpose** Organ culture as a storage method for human donor corneas allows a medium term storage period, evaluation of the endothelium by light microscopy and microbiological testing before grafting. It is a preferred storage method in Europe since the early eighties. Eye banks have adjusted the technique to their specific needs and experience resulting in differences in the procedure. The risks during exchange of tissue from different eye banks are evaluated.

**Methods** With the help of the EEBA Directory the technical details provided by the different banks are compared. Information about graft outcome is collected with respect to endothelial cell density after grafting, graft survival, adverse reactions such as primary graft failure.

**Results** The composition of the storage solutions differs slightly. The temperature ranges from 30 to 37°C. The survival of the corneal cells is good. The concentration of dextran used to reverse the swelling of the cornea varies from 4 to 8 % and the time the cornea spends in the transport solution is 1 to 10 days. The time schedule for the microbiological tests is different.The endothelium is inspected and selected with criteria based on cell density and morphology. The cut off points vary. All banks claim to have good clinical results, but reliable information about primary graft failure and keratitis/ophthalmitis caused by the donor tissue is lacking. Recent experience in the Netherlands suggests that the clinical outcome of tissue processed in different banks might be different when tissue is exchanged.

**Conclusion** The storage of donor corneas by organ culture facilitates the exchange of tissue with a well-defined quality and a medium term storage period. The differences in the procedure require normalization and assessment of the risks to meet the demands and expectations of surgeons.

## 2123
## Hypothermic storage (2-6 degrees Celsius)

*ZANETTI E*
*Fondazione Banca degli Occhi del Veneto, Venice*

**Purpose** Corneas must be stored in a preservation medium before surgery. The primary aim of corneal storage is the maintenance of endothelial viability from the time of corneal excision to transplantation.

**Methods** One of the two main methods for preserving the cornea is hypothermic storage (2-6 degrees Celsius) which use liquids deriving from a medium for cellular cultures.At 2-6°C the metabolic activity of endothelial cells is minimal and the endothelial pump cannot maintain corneal transparency. For this reason, the storage is performed in the presence of deturgescent agents that, when added to the media, maintain the corneal stroma poor in water and preserve corneal transparency for a few days. The most commonly used corneal storage media contain dextran and/or chondroitin sulphate as deturgescent agents. Storage liquids also contain antibiotics (gentamicin alone or in combination with stretomycin) that, together with the low temperature, prevent or limit bacterial growth. Commercially available solutions are also supplemented with a number of additives (energy sources, antioxidants, membrane stabilizing components, growth factors), but their specific contribution to the corneal storage has never been clarified.

**Results** A cornea stored at 2-6°C shows progressive cell degeneration, intercellular disruption, decreased cell adhesion to the Descemet membrane and death of the endothelium. Despite claims of serum-free media able to maintain donor corneas for up to 14 days without endothelial degeneration, the extent of endothelial loss during storage appears to be dependent on the biological quality of the tissue, and not on the composition of the medium.

**Conclusion** Thus the Fondazione does not store corneas in these solutions beyond 7-10 days, and most corneas are grafted after 3-5 days at 2-6°C.

## 2124
## Assessment of the endothelial cell density and morphometry during corneal storage: what do we measure and how?

*THURET G (1, 2), ACQUART S (3), GAVET Y (1, 4), GARRAUD O (3), GAIN P (1, 2)*
*(1) Lab 'Cell survival and adhesion in cancer and graft' EA3063, Faculty of medicine, Saint-Etienne*
*(2) Ophthalmology dpt, University hospital, Saint-Etienne*
*(3) Eye Bank, Etablissement Français du Sang Auvergne Loire, Saint-Etienne*
*(4) Ecole Nationale Supérieure des Mines de Saint-Etienne, Saint-Etienne*

**Purpose** Endothelial cell 'quality' is undoubtedly of paramount importance in the success of corneal graft. In eye banks, the endothelial cell density (ECD), and to a lesser extent, cell pleomorphism and polymegathism are the only surrogate parameters to estimate this quality, since no functional parameter is available yet. Several clinical studies indicate that higher the end storage ECD, longer is the graft survival. During organ culture, ECD and cell morphometry are determined through a light microscope after making cells temporarily visible following an osmotic preparation.The fixed and variable frame techniques for ECD determination will be described. Methods still routinely used in Europe will be discussed: several are manual either directly, in live, with observation through the microscope eyepiece, or using a printout of the picture and a grid overlay. Since a few years, computer-aided endothelial analysis is slowly gaining ground in the eye bank community. We will review the characteristics of the three commercially available systems with analysis of the experimental studies assessing these methods.Based on previous studies, we will discuss what should or could be the best method to assess the endothelial cell quality during storage with focus on the limitations of the manual count using the fixed frame technique. Computer-aided count is very likely to reduce the variability of the analysis. The importance of cell morphometry, objectively provided only by image analysis, will also be discussed.The assessment of ECD and morphometry requires reliable tools and methods that are now available. Nevertheless, considering the unique characteristics of this difficult tissue, the skill of technicians should also be carefully considered.

## 2125
## Immunological aspects of banking

*CLAERHOUT I*
*Department of Ophthalmology, Ghent University Hospital, Ghent*

**Purpose** To evaluate and discuss the immunological aspects involved in eyebanking.

**Methods** Three main topics will be adressed. For each of these, evidence based facts will be discussed. This will involve both an overview of animal studies as well as data from human clinical practice. The first deals with the effect of corneal storage on corneal immunogenicity. The second discusses the importance of HLA matching and finally the promising new prospect of immunologically altering the stored cornea through gene delivery is adressed.

**Results** Storage of corneal tissue results in migration of dendritic cells from the cornea into the culture medium. Recent evidence suggests that storage of corneal tissue also reduces the frequency of allograft rejection, especially in high risk patients. HLA matching, be it class I or II matching, seems to increase the chances of graft survival. Corneal storage permits adequate molecular typing methods to be performed. Donor corneas can be subject to gene transfer while in storage prior to transplantation. This make the cornea a unique model to try out gene therapy. However few studies have demonstrated highly efficient gene delivery and even fewer have managed to demonstrate any significant effect on graft survival.

**Conclusion** In this modern era, eye banking involves a lot more than just storing and delivering the donor cornea. It allows for time to perform adequate HLA matching and enables us to subject the donor cornea to different procedures to alter its immunological properties and thus possibly the outcome of corneal transplantation.

■ **2126**

## Influence of new lamellar techniques on eye bank activity

*MURAINE M*
*Ophthalmology, Rouen*

**Purpose** Deep lamellar keratoplasty (DLK) has recently been gaining popularity as an alternative option to penetrating keratoplasty in patients with stromal corneal pathologies. Main advantages of DLK are prevention of long-term endothelial loss, elimination of allograft reaction and short topical steroid treatment with less risk of glaucoma, cataract, infection or epithelial defects. As this technique allows surgeons to use corneal grafts with very low endothelial density, this type of surgical modification has a significant influence on Eye Bank activity. This aim of this presentation is to report such an influence.

**Methods** We reviewed our corneal graft activity at the Rouen University Hospital during a 42 months period and assessed the proportion of deep lamellar and penetrating keratoplasties. During the same period, we also evaluated our local Eye bank activity and registered the utilization of grafts with endothelium abnormalities only suitable for lamellar techniques.

**Results** Deep lamellar keratoplasty represented 29.8% of corneal transplantation in this center. Fifty two percent of corneas were suitable for penetrating keratoplasty. A further 36.6% of corneas were not suitable for endothelial deficiencies. Among these, 72.7% were used for deep lamellar keratoplasties and 27.3% were rejected. This permitted a 24.5% increase in corneal grafting activity. In contrast, Descemet's membrane was removed at the time of surgery in 11% of corneas with healthy endothelium which was used for deep lamellar keratoplasty.

**Conclusion** Diffusion of deep lamellar keratoplasty and close collaboration between Eye Banks and surgeons can promote a significant increase in corneal grafting. This could be a partial solution in countries confronted with corneal graft shortage.

■ 2131

## Effect of Bimatoprost, Latanoprost, PGF2a, Travoprost and Unoprostone in Quiescent Isolated Human Ciliary Arteries

*ALLEMANN RA*
*Laboratory of ocular Pharmacology and Physiology, Basel*

**Purpose** To investigate vasoconstrictive properties of the prostaglandin's analogues bimatoprost, latanoprost, prostaglandin F2a (PGF2a) travoprost, and unoprostone in isolated human ciliary arteries.

**Methods** In a myograph system (isometric force measurement), quiescent isolated human ciliary arteries were exposed either to bimatoprost, latanoprost, PGF2a, travoprost or unoprostone (0.1 nM – 0.1 mM) in the presence or in the absence of 1 µM of either SQ 29,548 or ICI-192,605 (TP-receptor antagonists). Time-control experiments without prostaglandin's analogues were run in parallel. Contractions were expressed as percent of 100 mM potassium chloride-induced contractions. Significance level was set to 5%. Ethanol was used to dissolve prostaglandin's analogues.

**Results** In comparison to time-control, bimatoprost, latanoprost, PGF2a, travoprost, and unoprostone induced significant contractions (0.1 mM: 65.8 ± 9.7, 89.3 ± 4.8, 72.4 ± 12.8, 47.8 ± 13.4, 56.5 ± 12.3, respectively). The latter were significantly inhibited either by SQ 29548 (20.8 ± 3.7, 25.5 ± 9.5, 10.6 ± 2.7, 11.0 ± 2.4, 7.0 ± 2.9, respectively) or ICI-192,605 (16.1 ± 7.6, 10.6 ± 3.1, 23.3 ± 7.1, 17.0 ± 6.8, 11.0 ± 6.0, respectively).

**Conclusion** In isolated human ciliary arteries, all prostaglandin's analogues tested induced contractions that were most pronounced with latanoprost and least marked with travoprost. These contractions could be inhibited by the TP antagonists SQ 29,548 and ICI-192,605.

■ 2132

## First report on a prostaglandin–ethanolamide (prostamide) receptor antagonist AGN 204396

*STRUBLE C (1), WOODWARD DF (2), KRAUSS AHP (2), NIEVES AL (2), PROTZMAN CE (2), WANG JW (2), BURK RM (3), DONDE Y (3), LANDSVERK K (1)*
*(1) Dept of Drug Metabolism, Covance Inc., Madison, WI*
*(2) Depts. of Biological Sciences, Allergan Inc., Irvine CA*
*(3) Depts. of Chemical Sciences, Allergan Inc, Irvine CA*

**Purpose** Prostaglandin (PG)–ethanolamides (prostamides) and PG-glyceryl esters are biosynthesized anandamide and 2-arachidonyl glycerol, respectively. Early studies of agonist effects of prostamide F2alpha and PGE2-glyceryl ester suggested that they are pharmacologically distinct from PGs. In the present studies, the prostamide receptor concept was evaluated using a selective antagonist. The feline iris sphincter provided an exacting test of the prostamide receptor hypothesis, since it is prostamide sensitive and contains functional prostanoid FP receptors.

**Results** In the feline iris sphincter, the prostamide antagonist AGN 204396 blocked the effect of prostamide F2alpha and its congener bimatoprost but did not antagonize the effects of PGF2alpha and prostanoid FP receptor selective analogs. To try the FP receptor counterpart experiment, the FP receptor antagonist AL-8810 was employed. AL-8810 behaved as a weak, full agonist in the feline iris and was unsuitable for antagonist studies. The prostamide antagonist AGN 204396 did not antagonize responses to AL-8810, thereby providing additional evidence that prostamide and prostanoid FP activity exist as distinct entities in the feline iris. AGN 204396 was not an antagonist at recombinant human prostanoid DP, EP1-4, FP, and IP receptors. AGN 204396 did not antagonize the iridial effects of PGE2-glyceryl ester.

**Conclusion** Studies in the feline iris demonstrated that AGN 204396 selectively blocks prostamide F2alpha and bimatoprost effects but not PGF2alpha and PGE2-glyceryl ester. The identification of an antagonist that selectively blocks prostamide effects provides further support for a novel prostamide-sensitive receptor.

■ 2133

## Polymorphism of the b-2 adrenoceptor and IOP lowering potency of topical timolol in healthy subjects

*FUCHSJÄGER-MAYRL GF (1, 2), MARKOVIC OM (1), LOSERT DL (1), LUCAS TL (1), SCHMETTERER LS (1, 3)*
*(1) Clinical pharmacology, Vienna*
*(2) Ophthalmology, Vienna*
*(3) Biomedical engineering and physics, Vienna*

**Purpose** Beta-2 adrenoceptor antagonists such as timolol are used in the treatment of glaucoma for more than 30 years. Several functionally important polymorphisms for the beta-2 receptor have been described. In the present study we hypothesized that a relation between the intraocular (IOP) lowering effect of timolol and beta-2 adrenoceptor polymorphisms may exist.

**Methods** A total of 270 healthy non-smoking subjects were screened and subjects with the homozygote mutant: Arg16/Gln27 (wild-type); the homozygote mutant: Gly16/Gln27 or the homozygote mutant: Gly16/Glu27 were included. In these subjects the IOP lowering effect of timolol was compared.

**Results** 24 subjects were included in the group Arg16/Gln27, 18 subjects in the group Gly16/Gln27 and 47 subjects in the group Gly16/Glu27. The ocular hypotensive effect of timolol was between 40 and 45% in all groups, but not significantly different between the three study groups (p= 0.979).

**Conclusion** The present study indicates that b-2 adrenoceptor polymorphism does not influence the ocular hypotensive effects of topical b adrenoceptor antagonists. Accordingly, other factors appear to be responsible for the intersubject variability seen with timolol in glaucoma subjects.

■ 2134

## Effects of topical clonidine and brimonidine on choroidal blood flow and intraocular pressure during isometric exercise

*WEIGERT G (1), RESCH H (1), GARHOFER G (1, 2), SCHMETTERER L (1, 3)*
*(1) Clinical Pharmacology, Vienna*
*(2) Ophthalmology, Vienna*
*(3) Center of Biomedical Engineering and Physics, Vienna*

**Purpose** Clonidine and brimonidine are both alpha 2 agonists that exert their effects on intraocular pressure (IOP) by causing both a decrease in aqueous humor production and an increase in uveoscleral outflow. Little is, however, known about potential vasoconstrictor effects of alpha-2 agonists in the posterior pole of the eye.

**Methods** We performed a randomized, double masked, two way cross-over study in 12 healthy male volunteers. Continuous laser Doppler flowmetry was performed during a 6 minutes squatting period to assess choroidal blood flow regulation during an increase in ocular perfusion pressure. Measurements were performed at baseline conditions, 60 minutes after instillation of either clonidine or brimonidine, and again after a resting period of 60 minutes. Systemic blood pressure was measured in 1 minute intervals and IOP was measured 3 times throughout the study.

**Results** Both substances induced a pronounced, but comparable (p=0.8) decrease in IOP. Isometric exercise increased mean arterial pressure and ocular perfusion pressure (p<0.01). This increase, however, was comparable between the clonidine and brimonidine study day (p = 0.88). Isometric exercise induced an increase in choroidal blood flow which was, however, less pronounced than the increase in ocular perfusion pressure. As compared to baseline the alpha-2 agonists decreased choroidal blood flow during isometric exercise (p = 0.0026) to a comparable degree (p = 0.86).

**Conclusion** Alpha-2 agonists may induce changes in choroidal blood flow even after single topical administration. Long-term studies are required to study potential long-term effects of brimonidine and clonidine.

■ 2135

## The influence of carbonic anhydrase inhibitors on cytoplasmic pH in nonpigmented ciliary epithelium

*DELAMERE NA (1, 2), TAMIYA S (1), MATHEWS SA (1), HOU Y (1)*
*(1) Ophthalmology and Vis. Sci., Louisville, KY*
*(2) Pharmacol. and Toxicol, Louisville, KY*

**Purpose** Carbonic anhydrase inhibitors (CAIs) reliably and effectively slow the rate of aqueous humor production. Although CAIs have been widely used for many years, questions still remain about their mechanism of action. CAIs reduce aqueous formation both in species with a net bicarbonate movement from blood to aqueous and species that exhibit net chloride movement but no net bicarbonate movement. Here, studies were conducted to examine effects of CAIs on cytoplasmic pH of cultured rabbit nonpigmented ciliary epithelium (NPE).

**Methods** Cytoplasmic pH was measured in cells loaded with the fluorescent dye BCECF. In some experiments, western blots were conducted to probe for the Src family of tyrosine kinases. Src activation was examined by probing for phospho-Src (p-Src).

**Results** In cells superfused with Krebs solution, the addition of the CAI dorzolamide to the superfusate caused a reduction in cytoplasmic pH detectable within 5 min. Acetazolamide, a different CAI, also reduced cytoplasmic pH. Since a change of cytoplasmic pH could influence cell signaling mechanisms, the activation of Src family tyrosine kinases was examined. Dorzolamide-treated cells displayed a marked change in p-Src western blot band density within 5 min.

**Conclusion** Carbonic anhydrase inhibition appears to elicit detectable changes of pH in cultured non-pigmented ciliary epithelium. It remains to be determined whether the altered Src activation observed in CAI-treated cells is linked to the observed pH change. The findings give rise to the idea that small changes of cytoplasmic pH may be sufficient to change some aspects of NPE function.

■ 2136

## Retinal Morphology after Intravitreal Administration of Erucylphosphocholine in an in-vivo Rat Model

*EIBL KE (1), SCHUETTAUF F (2), SHINODA K (2), MAY CA (3), WELGE-LÜßEN UC (1), KAMPIK A (1)*
*(1) Ophthalmology, Munich*
*(2) Ophthalmology, Tuebingen*
*(3) Anatomy, Dresden*

**Purpose** Erucylphosphocholine (ErPC) inhibits human retinal pigment epithelium (RPE) cell proliferation and contraction at non-toxic concentrations as previous in vitro studies have shown. Since this might represent a novel therapeutic option for the treatment of proliferative vitreoretinal diseases, the purpose of this first in vivo study was to evaluate posterior segment morphology of rat eyes after intravitreal administration of ErPC.

**Methods** Adult male Brown Norway rats were injected intravitreally with ErPC dissolved in BSS at a final concentration of 10 or 100 µM with BSS serving as control. Adverse effects on the anterior and posterior segment were assessed by slit lamp biomicroscopy and ophthalmoscopy. Morphology of posterior segments was evaluated by histology and retinal ganglion cell (RGC) quantification seven days after intravitreal administration of ErPC.

**Results** There was neither a statistically significant difference in the clinical examination nor in the number of RGC of treated versus control rats seven days after injection. Histologic sections of the posterior segment of 10 and 100 µM ErPC injected rats did not show any signs of retinal toxicity. On electron microscopic sections, there was no difference detected between the 10 µM and the control group. Only the 100 µM injected animals displayed a discrete irregularity of the Mueller cell and the retinal ganglion cell cytoplasm at the ultrastructural level.

**Conclusion** ErPC can safely be injected into the vitreous of adult rats at a concentration of 10 or 100 µM without toxic effects on the retina. This might be an important observation for ErPC as a novel treatment option for proliferative vitreoretinal diseases in the future.

## ▪ 2141
## Wellcome to RPE SIS

*UUSITALO H*
*Department of Ophthalmology, University of Kuopio, Kuopio*

**Purpose** Retinal pigment epithelium has a vital role in the regulation of the normal functions of retina and choroid. RPE cells are taking part in rhodopsin metabolism, taking care of the photoreceptor outer segment removal and degradation. They are protecting as a barrier the neuroretina and at the same time transporting nutrients and many other compounds e.g. drugs to it. RPE cells are regulating their environment and functions of the retina and choroid like angiogenesis by producing several cytokins. They are also an important part of the retinal wound healing process. The broad spectrum of functions of RPE cells in the normal retina make them key players in the pathogeneis of many retinal diseases like age related macular degeneration (AMD), and proliferative vitreoretinopathy. RPE cells are thus natural targets for drug and gene therapies and a potential cell type for retinal transplantation. The RPE SIS this year is focusing some of the most interesting aspects of RPE cells like lysosomal activity of RPE cells and lipofuscinogenesis, heat shock proteins, drug delivery and gene transfer and RPE cell repair and transplantation.

## ▪ 2142
## Lipofuscinogenesis and lysosomal dysfunction of RPE

*TERMAN A (1), BRUNK UT (2)*
*(1) Division of Experimental Pathology, Faculty of Health Sciences, Linköping University, Linköping*
*(2) Division of Pharmacology, Faculty of Health Sciences, Linköping University, Linköping*

**Conclusion** Lipofuscin (age pigment) is an intralysosomal, autofluorescent, polymeric material that accumulates progressively within aging long-lived postmitotic cells such as cardiac myocytes, neurons and retinal pigment epithelium (RPE). Lipofuscin forms as a result of iron-catalyzed oxidative modification of macromolecules within lysosomes and is neither degraded, nor exocytosed to any substantial extent. The main source of RPE lipofuscin are phagocytosed rod photoreceptor outer segments, although autophagocytosed material also somewhat contributes to its formation [1]. The role of oxidative stress in lipofuscinogenesis is supported by increased lipofuscin formation within RPE cells cultured under high (40%) ambient oxygen concentration or fed by pre-oxidized rod outer segments [2]. Heavy lipofuscin loading of RPE cells diminishes their functional capacity and decreases adaptability. In particular, lipofuscin-laden RPE cells show reduced phagocytosis and are increasingly sensitive to blue light irradiation [2, 3]. Lipofuscin accumulation within RPE cells is an important contributor to age-related macular degeneration, the main cause of blindness in elderly individuals [4]. References 1. Terman A, Brunk UT. Int J Biochem Cell Biol. 2004, 36: 1400-1404. 2. Nilsson SE, Sundelin SP, Wihlmark U, Brunk UT. Doc Ophthalmol. 2003, 106: 13-16.3. Boulton M, Rozanowska M, Rozanowski B, Wess T. Photochem Photobiol Sci. 2004, 3: 759-764.4. Kopitz J, Holz FG, Kaemmerer E, Schutt F. Biochimie. 2004, 86: 825-31.

## ▪ 2143
## Regulatory role of heat shock proteins in the ubiquitin proteolytic pathway in RPE cells

*KAARNIRANTA K*
*Department of Ophthalmology, Kuopio*

**Purpose** Pathogenesis of age–related macular degeneration (AMD) involves to impaired degradation of membranous discs shed from photoreceptor outer segments and accumulation of lysosomal lipofuscin in RPE cells. In addition to lysosomal protein degradation, plenty of cellular proteins are degradated in proteasomes. Prior to the proteolysis heat shock proteins (Hsps) tend to fold unfolded proteins due to their molecular chaperone capacity and thus prevent the accumulation of cytotoxic protein aggregates. Connection of Hsp90, Hsp70 and proteasome inhibition were evaluated in cellular protein aggregation in human RPE cells (ARPE–19).

**Methods** Amount and localization of Hsp90, Hsp70, ubiquitinated (Ub) proteins and microtubulins were analyzed by Western blotting and immunofluorescence analyses. Transmission electron microscopy was used to detect cellular organelles in ARPE–19 cells.

**Results** MG–132 proteasome inhibitor caused robust accumulation of Hsp70 protein and Ub protein conjugates in ARPE–19 cells. Electron microscopy analysis showed highly, in size and context, varied juxtanuclear protein aggregates in response to proteasome inhibition. The Hsp70 and Ub, but not Hsp90 colocalized with the protein aggregates. When the cells were subjected to Hsp90 inhibitor geldanamycin the amount of protein aggregates was clearly decreased together with increased disruption of microtubules. However, tubulin acetylation was not linked to the tubulin network disruption or protein aggregation.

**Conclusion** These data reveals the Hsps and ubiquitin–proteasome pathway association in control of protein turnover and aggregation in the RPE cells.

## ▪ 2144
## Barriers and mechanisms of non-viral gene delivery into retinal pigment epithelium

*URTTI A*
*Department of Pharmaceutics, University of Kuopio, Kuopio*

**Purpose** Retinal pigment epithelium (RPE) is an interesting target tissue for DNA delivery, a prerequisite in successful gene therapy. RPE phagocytoses rod outer segments, thereby having high capacity of internalisation of particulates, and furthermore transfected RPE might serve as secreting platform for many growth factors acting in neural retina or in retinal and choroidal vessels. We investigated gene transfer into RPE cells by several liposomal and polymeric carriers. RPE cell line was synchronized to various phases of cell cycle and then transfected with polyethylene imine (PEI) / DNA complexes. Both cellular uptake and transfection by DNA/carrier complexes was at lowest level when the cells are in the resting state suggesting that growth and division of cells facilitate DNA transfection. PEI/DNA complexes had poor transfection efficacy also in the polarized tight RPE cell monolayers that were cultured on filters. However, complexes of DNA with DOTAP/DOPE/protamine sulphate combination provided long lasting (2 months) secretion of marker gene product SEAP from such monolayers showing that non-dividing differentiated RPE can be transfected with non-viral systems. After intravitreal injection the DNA complexes are also in contact with the vitreous and neural retina before they enter the RPE. Our studies show that both vitreous and neural retina act as barriers in the gene transfer. Therefore, strategies to overcome these barriers are needed in order to obtain effective non-viral gene transfer to the RPE. Support: National Agency of Technology, Academy of Finland.

■ **2145**

## RPE repair and transplantation: the challenges for the future

*BOULTON M*
*Dept of Optometry and Vision Sciences, Cardiff University, Cardiff*

**Purpose** RPE dysfunction and death is a common feature of ophthalmic conditions such as AMD and retinitis pigmentosa. RPE transplantation offers one form of treatment. However, for successful transplantation surgery it is essential that donor RPE cells retain or achieve their in vivo phenotype once transplanted. The RPE is a single monolayer of cells which ensures survival of the overlying photoreceptor cells by performing a number of critical functions, i.e. maintaining the outer blood retinal barrier, transepithelial transport of nutrients and waste products, transport and storage of retinoids, phagocytosis of outer segment tips, protection against light damage and the synthesis of growth factors. Furthermore, the RPE also acts to regulate the integrity of the underlying choroidal capillaries. Both fresh (autologous and non-autologous) and cultured RPE cells have been transplanted into animals and patients with retinal degeneration. To date success has been limited and has been attributed to rejection, failure to attach to Bruch's membrane, cell damage, loss of RPE polarity and loss of differentiated characteristics. Furthermore, transplantation into a compromised and hostile subretinal space in diseased eyes will further reduce the likelihood of achieving the in vivo RPE phenotype. The possible reasons and limitation to RPE transplantation to date will be discussed, as will potential avenues (e.g. support matrices, ex vivo gene therapy) for improving outcome.

■ 2151

## Introduction : emerging agents and novel aspects in inflammatory eye diseases

*HERBORT CP (1, 2)*
*(1) Inflammatory Eye Diseases, La Source Eye Centre, Lausanne*
*(2) University of Lausanne, Lausanne*

**Purpose** This SIS deals with new findings in well known infectious or inflammatory eye diseases that lead to a new perception of these conditions as well as new disease entities. Fuchs' uveitis that is usually classified in the group of anterior uveitides, whereas we found that involvement of the posterior segment was preponderant including the unreported finding of disc hyperfluorescence present in virtually all cases. An old entity, tuberculous uveitis or presumed tuberculous uveitis, has been neglected in the past decades leading to underdiagnosis of the condition. It appears that this disease is reemerging and should be taken into account in the differential diagnosis of chronic granulomatous uveitis with a high therapeutic threshold for antiinflammatory agents. Clinical findings of so far unrecognized or ill-known ocular disease entities have been reported recently; this is the case for reckettsial infections and infections caused by the West-Nile virus. On the other hand, old commensal agents such as herpes viruses have been recognized to cause new forms of diseases. The workshop will close with a review of rare causes of infectious ocular inflammation.

■ 2152

## Neglected and unrecognized signs in Fuchs' uveitis

*HERBORT CP (1, 2), BOUCHENAKI N (1)*
*(1) Inflammatory Eye Diseases, La Source Eye Centre, Lausanne*
*(2) University of Lausanne, Lausanne*

**Purpose** Analyse anterior and posterior clinical signs in a series of Fuchs' uveitis seen at La Source Eye Centre and compare them to the data in the literature.

**Methods** Retrospective study of a group of patients diagnosed as Fuchs' uveitis in La Source Eye Centre from 1995 to 2004.

**Results** Between 1995 and 2004, 80 of a total of 928 new cases were diagnosed as Fuchs' uveitis (8.62%). Posterior involvement mostly in the form of a vitritis was seen in 78/80 cases (97.5%), representing the single most frequent sign before typical keratic precipitates seen in 96.25%. 21 patients (26.25%) had undergone fluorescein angiography, either because the diagnosis had not been made or to rule out another cause of uveitis. In all patients but one the optic disc on the Fuchs' side was hyperfluorescent. only one patient did not show any disc hyperfluorescence because this papilla was partially atrophique after a traumatic avulsion of part of the optic disc. Hyprfuorescence was not related to the amount of vitritis present.

**Conclusion** The posterior signs are more frequent than the anterior involvement in Fuchs' uveitis. This is often ignored by the literature and is one of the causes of the mismanagement. Papillary hyperfluorescence present in practically all cases on the eye of Fuchs' uveitis has not been described before to the best our knowledge. These findings represent an additional argument, indicating that Fuchs' uveitis seems to be an inflammatory disease rather than a degenerative disease.

■ 2153

## Tuberculous uveitis, a re-emerging cause of uveitis

*CIMINO L (1), HERBORT CP (2, 3), SALVARANI C (4), BOIARDI L (4)*
*(1) Ocular Immunology Unit, Ospedale S.M. Nuova, Reggio Emilia*
*(2) Inflammatory Eye Diseases, La Source Eye Center, Lausanne*
*(3) University of Lausanne, Lausanne*
*(4) Rheumatology Unit, Ospedale S.M. Nuova, Reggio Emilia*

**Purpose** Over the last decade ocular involvement due to tuberculosis has re-emerged. Here we report a group of patients with presumed tuberculous uveitis.

**Methods** Criteria for presumed tuberculous uveitis included the presence of a hyperpositive tuberculin skin test with compatible uveitis and the exclusion of other possible etiologies. We recorded data about patient age, sex, medical history, results of the ophthalmological examination, angiographic aspects, results of laboratory work-up and systemic and local drug therapy.

**Results** 37 patients with presumed tuberculous uveitis were included in the study. A specific antituberculous therapy was given for a minimum of 6 to a maximum of 24 months. The therapy resulted in a highly significant increase in visual acuity ($p<0.001$), a highly significant decrease of recurrences, with only three recurrences observed during the follow-up, and a highly significant decrease in intra-ocular pressure ($p<0.001$). Extraocular involvement was found in over half of the patients (59%).

**Conclusion** Our study confirms the existence of tuberculous uveitis and supports the validity of the proposed diagnostic criteria. Ignorance of the diagnosis leads to inappropriate, ineffective and potentially dangerous long-term corticosteroid and/or immunosuppressive therapies. Recognition of the correct diagnosis and specific therapy avoids recurrences, improves visual acuity and intra-ocular inflammation, and decreases intra-ocular pressure.

■ 2154

## Ocular lesions in rickettsial and West Nile virus infections

*KHAIRALLAH M*

**ABSTRACT NOT PROVIDED**

■ **2155**

## New manifestations of ocular herpetic diseases

*BODAGHI B*
*Ophthalmology, Paris*

**Purpose** New viral entities have been recently included in the previously well-described group of viral ocular diseases. Among this wide spectrum of diseases we will especially present the intraocular involvement due to different members of the Herpesviridae family. Diagnostic confirmation is a major issue and allows specific therapeutic strategies based on antivirals and corticosteroids. Therefore, it could be possible to prevent major relapses.The field of viral-induced anterior uveitis has been recently revisited by using molecular techniques. Several pitfalls, such as false positives, have been observed with PCR, underlining the necessity to interpret the results obtained with caution. The development of new techniques such as real-time PCR offers several advantages in comparison to conventional PCR, including speed, simplicity, reproducibility, quantitative capability and low risk of contamination.CMV-associated hypertensive anterior uveitis in immunocompetent hosts represents a new entity and preliminary results seem also to identify this virus as the causative agent of Posner-Schlossman syndrome. On the other hand, serological testings remain important in different clinical situations such as Fuchs heterochromic cyclitis, which may be associated with rubella virus infection, member of another viral family. Other agents, such as HSV-1, 2, VZV, EBV and HHV-8 are involved in different forms of nonnecrotizing herpetic retinopathies. When available, antivirals should be used to control ocular inflammation but immunomodulators such as interferon alpha are useful to control HHV-8 or EBV-induced uveitis.

■ **2156**

## How to manage and diagnose intraocular inflammatory oddities

*DE SMET M*
*Ophthalmology, University of Amsterdam, Amsterdam*

**Purpose** To describe an approach to the diagnosis and management of non malignant mascarade.

**Methods** A comprehensive literature review of the past 5 years in PubMed was performed to identify unusual or emerging cases of inflammatory, traumatic, or toxic uveitis affecting the anterior or posterior segment of the eye.

**Results** Several unusual forms of uveitis have recently been identified. These include bcg induced uveitis/endophthalmitis, West nile virus affecting the retina, Dengue fever in travellers returning from Asia, and a recurrence of subacute sclerosing pan encephalitis. Beta hemolytic streptococcus was recently associated with papillophlebitis and is known to cause multiple serous retinal detachments. Cilia of human or caterpillar origin as well as hairs from specific arachnid species are associated with ophthalmia nodosa. Poor instrument cleansing particularly of phaco instrumentation has been associated with post-operative infections, while the persistence of detergent in silicone tubing or cannulas has been associated with an acute toxic endophthalmitis-like inflammation.

**Conclusion** Awareness of various unusual uveitic and pseudo-uveitic conditions can facilitate identification of the cause in patients with unusual presentations.

■ 2161

## Characterization of the human extraocular muscles at the cellular and molecular level

*PEDROSA DOMELLOF F (1, 2), KJELLGREN D (2), THORNELL L-E (1)*
*(1) Dept of Integrative Medical Biology, Anatomy, Umeå*
*(2) Dept of Clinical Sciences, Ophthalmology, Umeå*

**Purpose** To present an overview of the composition of the human EOMs with respect to proteins determining contraction and relaxation properties; extracellular matrix and cytoskeleton.

**Methods** Human EOMs were studied with immunocytochemistry, using antibodies specific against several slow and fast myosin heavy chain (MyHC) isoforms, SERCA-1 and -2, slow and fast myosin binding protein C (MyBP-C), laminin chains alpha 1-5, beta 1-2 and gamma-1, desmin, nestin, synemin, dystrophin, utrophin, plectin, vinculin, spectrin, dystrobrevin, dystroglycans, sarcoglycans, nitric oxide synthase, nebulin, titin, myotilin, alpha-actinin and acetylcholinesterase.

**Results** The hallmark of the human EOMs was a remarkable heterogeneity in the levels of contractile and calcium pump proteins. The fibers had complex MyHC contents (22 distinct MyHC combinations) but could be divided into 3 major groups. The majority of the fibers contained both SERCA isoforms. There was no co-ordination between MyHC and MyBP-C composition. Laminin chains alpha-4 and 5 were present in addition to those found in limb muscle. The human EOMs showed substantial differences in cytoskeletal protein composition between the fibers in the orbital and the global layer. The fiber cytoskeleton composition was heterogeneous, in particular with respect to dystrophin, dystrobrevin, desmin, nestin and synemin and more in the global than in the orbital layer.

**Conclusion** The fibers of the human EOMs have a unique molecular portfolio that reflects i) a wide continuum of contraction and relaxation properties and ii) a different organization of the extracellular matrix and of the cytoskeleton, which may be of importance for the particular responses of the EOMs to neuromuscular disease.

■ 2162

## The role of innervation in the development of extraocular muscles

*PIEH C*
*Freiburg*

**ABSTRACT NOT PROVIDED**

■ 2163

## Sensory receptors in extraocular muscles (EOM) and their potential role in oculomotor control

*HAUGEN I-B K, BRUENECH JR*
*Biomedical research unit, Dep. of optometry and visual science, Buskerud University College, Kongsberg*

**Purpose** Whether or not EOM proprioception contributes to spatial stability has been long debated, a controversy partly sustained by the reported disparities in receptor complement between species. One of the insufficiencies in fully comprehending oculomotor control is the limited knowledge of proximal tendon receptors. The aim of this study was hence to increase the pool of data regarding apical nerve terminals in a wide span of species and thereby contribute to the comparative picture of receptor populations.

**Methods** Superior rectus (SRM) and levator palpebrae superioris (LPS) muscles were obtained from 6 mature sheep, monkeys, cats, rabbits, guinea pigs and rats. Tissues were prepared following standard histochemical techniques for light and electron microscopy.

**Results** Proximal Golgi tendon organs (GTO) and myotendinous cylinders (MTC) were confirmed present in sheep and cat SRM, as well as GTO in sheep LPS. Apical nerve endings were exposed in SRM of monkey, rabbit, guinea pig and rat. They had a patchy distribution and derived from axons approaching tendon through muscle. All terminals were incompletely lined by fibrocytes, with exception of those in rat. Neuromuscular contacts were exposed in monkeys. Substantial variations with regard to shape and density were found between species and animals. GTO were not observed.

**Conclusion** The distribution of apical nerve terminals in typical receptor areas, absence in LPS, indications of encapsulation and resemblance to MTC, are suggestive of an afferent origin. The low density and inconsistency in distribution between animals imply that they provide a minimal and highly variable sensory contribution. Comparison between novel and known receptor data will be made and clinical implications discussed.

■ 2164

## The structural organization of the distal insertions of human extraocular muscles (EOM)

*BRUENECH JR, KJELLEVOLD HAUGEN I-B*
*Biomedical research unit, Dep. of optometry and visual science, Buskerud University College, Kongsberg*

**Purpose** Recent studies have promoted the concept of a double insertion of extraocular rectus muscles, one part attaching the globe and the other on muscle pulley/sleeve (Demer et al., 2000; Ruskell et al., 2005). The muscle fibres in the respective insertions are argued to have different functions, yet their morphological features are still unclear. The aim of this study was hence to revise the organization of the distal insertion of human EOM, with emphasis on muscle fibre morphology and neuromuscular architecture.

**Methods** Cadaver materials from previous studies were re-examination and transverse sections of medial and temporal rectus muscles from 7 new subjects (4-91 years) analysed. None of the subjects had any history of neuromuscular disease or binocular anomaly. Tissues were prepared for light and electron microscopy by standard histochemical techniques.

**Results** Fibrillenstruktur and Felderstruktur fibres were identified in the myotendinous regions of both insertions. The insertions were interconnected by an abundance of connective tissue. Features usually associated with muscle fibre atrophy were observed in the samples from the older subjects.

**Conclusion** The current study confirms the previously described double insertion of EOMs. In addition, both insertions were found to hold Fibrillenstruktur as well as Felderstruktur fibres. The notion that these fibres can manipulate the insertion-angle during eye rotation cannot be dismissed. However, the substantial amount of connective tissue present between the insertions would arguable oppose the freedom of movement, which is a fundamental factor in the active pulley-hypothesis. The observed age-related changes arguably imply the potential effect of the double insertion to decline with age.

## ▪ 2211
## Inhibitory VEGF isoforms in Diabetic Retinopathy

*BATES DO*
*Microvascular Research Laboratories, Department of Physiology, University of Bristol, Bristol*

**Purpose** VEGF controls pathological angiogenesis and permeability in diabetic retinopathy and age-related macular degeneration. The inhibitory splice variant VEGF165b is anti-angiogenic in vivo. To determine the role of VEGF165b in eye disease, we investigated its expression and role in human ocular tissues and retinal pigment epithelial (RPE) cells.

**Methods** Ocular tissues were dissected from human eyes and RPE cells isolated and characterised by immunohistochemistry with an anti-cytokeratin antibody. Western blotting and isoform-specific ELISA were used to examine VEGF protein expression.

**Results** Multiple isoforms of VEGFxxxb protein eg VEGF121b VEGF189b, VEGF183b, VEGF145b and large VEGFb isoforms were detected in the lysate of RPE cells, retina, iris, lens, sclera and vitreous, and confirmed to be VEGF isoforms. Moreover, VEGFxxxb isoforms were relatively downregulated in the vitreous of diabetic patients. Hypoxia induced upregulation of secreted levels of total VEGF in conditioned medium, determined by ELISA, but did not increase VEGFxxxb concentration. IGF was also able to increase total VEGF expression by these cells, whereas it downregulated VEGFxxxb isoforms. Moreover, this splicing switch could be mimicked by transfection of RPE with splicing factors. We will also demonstrate the effect of VEGF165b injection in mouse models of proliferative retinopathy.

**Conclusion** Human ocular tissues and RPE express multiple VEGFxxxb splice variants, which are regulated by environmental conditions, and the balance of stimulatory and inhibitory isoforms may play a significant role in the development of angiogenic eye diseases, and may be therapeutically useful. Funded by Diabetes UK.

## ▪ 2212
## Molecular therapies for ocular neovascularisation

*ALI R*
*London*

**ABSTRACT NOT PROVIDED**

## ▪ 2213
## Systemic Bevacizumab (Avastin®) Therapy for Neovascular Age-Related Macular Degeneration (SANA) Study

*MICHELS S (1), SCHMIDT-ERFURTH U (1), ROSENFELD PJ (2)*
*(1) University Eye Hospital Vienna, Vienna*
*(2) Bascom Palmer Eye Institute, Miami*

**Purpose** To evaluate the treatment effects observed following systemic bevacizumab (Avastin®, Genentech Inc.) therapy in patients with age-related macular degeneration (AMD) and subfoveal choroidal neovascularization (CNV).

**Methods** This open-label, uncontrolled clinical study enrolled AMD patients with subfoveal CNV. Patients were treated initially with 2 or 3 infusions of bevacizumab (5mg/kg) at 2 week intervals. At baseline and at each follow-up visit, patients underwent a medical review, ETDRS visual acuity (VA) assessment, ophthalmologic examination, and optical coherence tomography imaging. Angiographic assessment of the CNV was performed every 4 weeks.

**Results** Of the 18 patients enrolled in the study, 11 patients have been followed for at least 6 months. Visual acuity improvements were evident 1 week after the first dose. Angiographic leakage from CNV was markedly reduced or eliminated by 4 weeks. At 6 months, the median and mean visual acuity letter scores in the study eyes increased by 16 letters (p=0.007) and 13 letters (p=0.001), respectively, and the median and mean OCT central retinal thickness measurements in the 11 study eyes decreased by 117 microns (p=0.008) and 128 microns (p=0.002). Only 1 of the 11 patients followed through 6 months received retreatment prior to the 6 month visit. Mild hypertension was the only treatment-related adverse event identified.

**Conclusion** After 6 months, bevacizumab therapy resulted in stable or improved visual acuity with a corresponding decrease in OCT central retinal thickness and angiographic leakage from CNV. The therapy was well tolerated. In addition, these results suggest that bevacizumab may have better durability than similar drugs injected intravitreally.

## ▪ 2214
## A Phase II Randomized, Double-masked Trial of Pegaptanib, an Anti-Vascular Endothelial Growth Factor Aptamer for Diabetic Edema

*ADAMIS T*
*New-York*

**Purpose** To evaluate the safety and efficacy of pegaptanib in the treatment of diabetic macular edema (DME).

**Methods** Design: A randomized, double-masked, multicenter, dose-ranging, controlled trial. Intervention: Intravitreous pegaptanib (0.3 mg, 1 mg, 3 mg) or sham injections at study entry, week 6, and week12 with additional injections or focal photocoagulation as needed for another 18 weeks. Final assessments were conducted at week 36.Main Outcome Measures: Best-corrected VA; central retinal thickness; need for photocoagulation.

**Results** 172 patients were studied. Median VA was better at week 36 with 0.3 mg (20/50) compared with sham (20/63) (P=.04). A larger proportion of those receiving 0.3 mg gained VA: ≥10 letters (approximately 2 lines): 34% vs. 10% (P=.003); ≥15 letters: 18% vs. 7% (P=.12). A larger proportions of those receiving 0.3 mg versus sham had an absolute decrease of both ≥100 microns (42% vs. 16%; P=.02) and ≥75 microns (49% vs. 19%; P=.008). Photocoagulation was deemed necessary in fewer subjects in each pegaptanib arm (0.3 mg vs. sham, 25% vs. 48%; P=.04). All pegaptanib doses were well tolerated. Endophthalmitis occurred in 1/652 injections (0.15%/injection, i.e., 1 [0.8%] of 130 pegaptanib subjects).

**Conclusion** In this Phase II trial, patients treated with pegaptanib had better VA outcomes and were more likely to show reduction in central retinal thickening, and deemed less likely to need photocoagulation at follow up.

## ▪ 2221
## Corneal wound healing and blood derived growth factors

*DIGHIERO PLD*

**Purpose** To report the effect of topically applied autologous blood derived growth factors on corneal wound healing.

**Methods** Platelet gel, platelet rich plasma, and autologous serum and were prepared from patient's blood at the blood transfusion laboratory of the University of Poitiers teaching hospital.Platelet gel was harvested by centrifugating 140 ml of blood at 2400 rpm for 10 min, and separating platelet rich plasma (PRP) from platelet poor plasma (PPP). Autologous thrombin is added to PRP and 20ml of platelet gel is obtained. In order to obtain platelet rich plasma concentrates, PRP + PPP were centrifuged at 3600 rpm for 15 min, and then the newly obtained PRP fraction was recentrifuged at 3600 rpm for 15 min resulting in concentrating platelets up to 1 million/µl.Autologous serum was obtained by centrifugating 100ml of blood at 3000 rpm for 15 minutes. It was separated and diluted 1:4 in saline.

**Results** Optimized techniques for harvesting platelet gel, platelet rich plasma, and autologous serum can be performed in conventional blood transfusion laboratories. Corneal growth factors play a major physiological role in activating signaling cascades regulating wound healing. Topical growth factors enhanced corneal healing when applied to the wounded cornea in acute and chronic epithelial ulcerations, and in ocular surface burns with limbal stem-cell deficiency.

**Conclusion** Topical autologous blood derived growth factors might become a new cost-effective tool for the management of ocular surface disorders, and for ocular surface reconstruction.Preparation from the patient's own blood and reported preliminary clinical studies results plead for the safety and efficiency of topical autologous blood derived growth factors.

## ▪ 2222
## Amniotic Fluid and Ocular Surface Disease

*BEHRENS A*
*Ophthalmology, The Wilmer Ophthalmological Institute, Baltimore, MD*

**Purpose** To evaluate the efficacy of topical human amniotic fluid (HAF) in the treatment of acute alkali burns of the eye in a murine model.

**Methods** A chemical burn using 2 µl of NaOH 0.15 M was created in the right eye of 30 mice. Animals were then divided in three groups according to the topical treatment administered: pre-term HAF (group 1, n=10), term HAF (group 2, n=10), and saline (group 3, n=10/control). Treatment was applied 5 times/day for the first week, and 3 times/day for another week. Digital photography was taken and percentage of epithelial defect was measured on days 2 and 4. The degree of ocular damage was semi-quantitatively assessed. On day 14, eyes were enucleated and histology was performed.

**Results** Median epithelial defect (IQR 25th, 75th percentile) at day 4 was 9.93% (8.57, 11.27) for group 1, 7.30% (5.96, 8.97) for group 2, and 18.92% (11.71, 27.64) for the control group ($p<0.0076$). The overall change (difference in slope) in ocular burn score between days 2 and 14 was -0.127 ($p=0.009$) in groups 1 vs. 3, -0.134 ($p=0.012$) in groups 2 vs. 3, and 0.007 ($p=0.88$) in groups 1 vs. 2. Histologically, saline treated corneas showed more inflammatory cells and blood vessels than the HAF treated corneas.

**Conclusion** Topical HAF was effective in the treatment of acute alkali burns in our model, limiting the degree of ocular damage and promoting re-epithelialization when compared to isotonic saline.

## ▪ 2223
## Ocular surface defense and innate immunity

*KINOSHITA S*
*Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto*

**Results** Ocular surface epithelium is the key first-line defense as a part of the mucosal innate immune system against pathogens. The ability of these cells to recognize microbial motifs and pathogen-associated molecular patterns (PAMPs) rests on pattern recognition receptors (PRRs) including toll like receptors (TLRs). Among them, TLR2, TLR3, and TLR4 exist in ocular surface epithelium, especially TLR3, inducing the secretion of inflammatory mediators. Conversely, a novel IkB protein, IkBzeta/MAIL, present in ocular surface epithelium, regulates negatively the pathological progression of ocular surface inflammation. Thus, the innate immune response of ocular surface epithelium is distinct from that of immune-competent-cells, which implies the symbiotic relationship between ocular surface epithelium and commensal bacteria inhabiting the ocular surface. The corneal stroma possesses keratocytes, many of which originate from BM-derived cells. Since some of these cells are BM-derived antigen-presenting cells, such as dendritic cells and macrophages, their function is presumably critical for defending cornea from microbes and keeping the cornea transparent.

## ▪ 2224
## AMT benefits on corneal wound healing

*DUA HS, HOPKINSON A*
*Ophthalmology and Visual Sciences, University Hospital, Nottingham*

**Purpose** To review the benefits of Amniotic membrane transplantation on corneal wound healing in the light of recent scientific evidence.

**Methods** Critical evaluation of the literature and histopathology (light, electron and immunocytochemistry) and proteome analysis of amniotic membrane from different donors.

**Results** The amniotic membrane is used extensively in ophthalmic surgery. For many indications it has proved to be quite useful but the exact mechanism of action of the membrane for several clinical applications is not known. Its mechanism is inferred from its chemical constituents but recent evidence from our and other laboratories has shown that there are several inter and intra membrane variations that can confound the effects of the membrane. This aspect is well illustrated with the analysis of TGF beta in different membranes. The membrane supports the migration and adhesion of epithelial cells allowing stratification. Its most consistent mode of action relaltes to its ability to act as a substrate. Keratocytes can migrate through breaks in Bowman's membrane and repopulate the amniotic stroma. These cells are largely fibroblasts and myofibroblasts as shown by immunohistochemistry

**Conclusion** Proper standaradisation of the membrane and consistent criteria of succes and failure are required before its efficacy can be fully evaluated. Besides acting as a substrate for epithelial cell migration and adhesion, the membrane can also provide the scaffold for stromal keratocytes to migrate into and build new 'scar' tissue to strengthen areas of corneal thinning and melts.

■ 2225

## Early AMT in severe bacterial keratitis: our 2 years experience

*GICQUEL JJ, ELLIES P, MERCIE M, DIGHIERO PLD*
*Ophthalmology / CHU Jean Bernard, Poitiers*

**Purpose** To determine whether a combination of early amniotic membrane transplantation (AMT) and early topical steroid treatment could be a safe adjuvant therapy for relieving pain, promoting healing, and limiting scarring, in severe bacterial keratitis.

**Methods** We studied a prospective, non-comparative series of 19 cases of severe acute bacterial keratitis treated by immediate maximal topical antibiotics followed at 48 hours by AMT plus topical steroid treatment. All patients underwent corneal scraping, before receiving any medication. Only those with positive bacterial direct examination were included. Single-layer epithelial side-down or multilayer epithelial side-up transplants were performed. The main outcome measurements were pain relief (using the NRS-11 numeric rating pain scale) and healing of the corneal epithelium.

**Results** The mean follow-up period was 14,42 months (6 to 23 months), with AMT performed once in 4 patients and twice in 15 patients. Gram negative germs were present in 67% of cases. A significant drop from a mean 8,17 (sd 0,94) pain score, on the admission day, to a mean 1,83 (sd 0,58) shortly after surgery was noted. Epithelial healing was achieved between 10 and 37 days (mean 16,5 days (sd 7,97)). Neither perforation nor conjunctivalization was observed.

**Conclusion** Our results show that early AMT combined with high-dose topical steroid application is a safe and efficient procedure, and gives immediate pain relief, in severe bacterial keratitis.

■ 2226

## The potential of hair follicle stem cells in corneal reconstruction

*BARRANDON Y*

**ABSTRACT NOT PROVIDED**

■ 2227

## Scleral Contact Lenses in the management of Ocular Surface disorders

*MURAINE M (1), DELCAMPE A (1), LAROCHE JM (2), DOAN S (2), HOANG XUAN T (2), BRASSEUR G (1)*
*(1) Ophthalmology, Rouen*
*(2) Ophthalmology, Paris*

**Purpose** to describe our experience in the use of gas-permeable scleral contact lenses in patients with severe corneal surface diseases.

**Methods** In this prospective study, 42 eyes of 24 patients with ocular complication after Stevens-Johnson and Lyell syndromes (20 patients), chemical burn (3 patients), exposure keratitis (1 patient) were fitted with an extended-wear gas-permeable scleral lens. We used a copolymer of metacrylates and fluorine comonomer (Contamac°, USA). Lissamin Staining, Fluorescein staining, Photography of the ocular surface was performed in each cases. Visual acuity, symptoms of ocular discomfort and the Ocular Surface Disease Index were noted.

**Results** The mean age of the 24 patients was 34 years (range 25 to 65 years). Follow up was 10 months. Diameters of scleral lens in our study ranged from 16 to 19 mm. The functionality of tear-fluid interchange was confirmed in all cases by observing the passage of fluorescein dye from the fornix into the fluid compartment.Improvement in best-corrected visual acuity (defined as a gain of 2 or more Snellen lines) was observed in 31 eyes (74%), 22 patients (91.6%) report a marked relief of ocular discomfort, 19 patients (79%) noted a marked reduction in photophobia with the use of gas-permeable scleral contact lenses. Twenty three (95%) of the 24 patients reported improvement in their quality of life.

**Conclusion** We too now find that gas-permeable scleral contact lens wear is an additional effective strategy in the management of selected patients with severe ocular surface disorders.

■ 2228

## Corneal wound healing and growth factors

*RIECK P*

**Purpose** A multitude of medical and surgical measures are available to modulate corneal wound healing. This presentation illustrates the long way of peptide growth factors from the hopeful basic sciences results to the sometimes disappointing outcome of clinical studies.

**Methods** Most of the growth factors with potential wound modulating effect (EGF, FGF, KGF, NGF, TGFß, PDGF) have been tested primarily in vitro in cultures of corneal cells from different origin. The assays used were based on cellular proliferation, migration, adhesion and cytotoxicity. In addition, the effect of exogenously applied growth factors has been evaluated in different wound models in animals.Finally, preparations of growth factors that were effective in animal studies have been tested in clinical trials, mainly concerning the modulation of epithelial healing.

**Results** The dissemination of corneal refractive surgery has largely enhanced our knowledge on corneal wound healing. Growth factors play a role after stromal removal, in corneal neovascularization and corneal (re-)innervation. It has become evident that epithelial-stromal interaction and the role of growth factors in keratocyte apoptosis is of major importance for the wound healing process. However, up to now no significant wound healing effect could be shown in clinical trials with topical administration of growth factor preparations.

**Conclusion** The disappointing clinical studies with exogenous pure growth factors demonstrate that a thorough understanding of the mechanism and function of growth factors is required to ameliorate targeting of these factors in therapeutic ophthalmic applications. A positive effect of growth factors on corneal wound healing is evident from the success of amniotic membrane transplantation and autologous serum application.

## ▪ 2231
## Retinal oximetry: Comparison of data from two centers

*HARDARSON SH (1), KARLSSON RA (2), HALLDORSSON GH (2), KAGEMANN LE (3), RECHTMAN E (3), ZOEGA GM (1), EYSTEINSSON T (1), BENEDIKTSSON JA (2), THORSTEINSSON A (4), BEACH JM (5), HARRIS A (3), STEFANSSON E (1)*
*(1) Ophthalmology, University of Iceland, Reykjavik*
*(2) Electrical- and Computer Engineering, University of Iceland, Reykjavik*
*(3) Ophthalmology, Indiana University School of Medicine, Indianapolis, IN*
*(4) Anesthesiology, University of Iceland, Reykjavik*
*(5) Stennis Space Center, Mississippi*

**Purpose** To measure hemoglobin O2 saturation (SO2) in retinal vessels and to test the sensitivity of an automatic spectrophotometric oximeter, using data from two centers.

**Methods** Specialized software automatically identifies the retinal blood vessels on fundus images, which are obtained with 4 different wavelengths of light. The software calculates optical density ratios (ODRs) for each vessel. Venous SO2 can be calculated assuming the relationship $\Delta SO2 = k\Delta ODR$ and using finger pulse oximetry values for retinal arterioles. Healthy volunteers inhaled 12% (n=4), 21% (n=17) or 100% (n=17) oxygen and the results were used for calibration (n=5) and examination of sensitivity (n=17). 5 subjects were measured in Reykjavik (16 bit digital camera) and 12 in Indianapolis (8 bit digital camera).

**Results** The calculated slope was k= -89% (-125 to -53%, mean and 95% c.i., linear regression). During normoxia, the venous SO2 for pooled data (n=17) was 72±10% (mean±SD); 72±11% for the Indiana images (n=12) and 73±5% for the Reykjavik images (n=5). The corresponding figures during 100% O2 breathing are 86±9% (n=17), 86±11% (n=12) and 88±6% (n=5). The difference between normoxia and hyperoxia is significant ($p<0.0001$, paired t-test, n=17). SO2 will be displayed on pseudocolor fundus images.

**Conclusion** The retinal oximeter is sensitive to changes in venous SO2 when concentration of O2 in inhaled air is changed. The similarity of the results from two centers using the same system except for 8 bit and 16 bit digital cameras suggests that the method is fundamentally reliable.

## ▪ 2232
## Altered retinal blood flow regulation in response to hyperoxia during experimental endotoxemia in healthy humans

*POLSKA E (1), KOLODJASCHNA J (1), SCHMETTERER L (1, 2)*
*(1) Clinical Pharmacology, Medical University of Vienna, Vienna*
*(2) Center for Biomedical Engineering and Physics, Medical University of Vienna, Vienna*

**Purpose** It has been shown that diabetic retinopathy and HIV-related retinopathy are associated with altered retinal vasoconstrictor responses to hyperoxia. In the present study we investigated whether an endotoxin infusion may alter the retinal vasoconstrictor response to hyperoxia to elucidate whether retinal vascular dysregulation is present in a model of inflammation in humans.

**Methods** 16 healthy male volunteers participated in this randomized, placebo controlled two-way cross over study. On each study day two 100% oxygen inhalation periods were scheduled. The first was performed before drug administration and the second 480 minutes after administration of either Lipopolisacharide (LPS) or placebo. Retinal vessel diameters were studied with the Retinal Vessel Analyzer and retinal red blood cell velocity with laser Doppler velocimetry. Based on these measurements retinal blood flow was calculated. In addition, the blue field entoptic system was used to assess white blood cell (WBC) velocity, density and flux in the perimacular region.

**Results** LPS significantly increased perimacular WBC density ($p<0.05$) indicating an increase in the number of circulating leukocytes after LPS administration. As expected systemic hyperoxia induced a pronounced decrease in retinal blood flow before drug administration ($p<0.05$). This response was not altered by placebo administration, but was significantly reduced after LPS administration ($p<0.05$).

**Conclusion** These data indicate that retinal blood flow regulation in response to hyperoxia is altered in the human LPS-model of inflammation. Accordingly, retinal vascular dysregulation in diabetic retinopathy and HIV retinopathy may be related to inflammatory processes in these diseases.

## ▪ 2233
## Breathing of 100% oxygen does not alter the response of retinal vessels to stimulation with flicker light

*GARHOFER G (1, 2), WEIGERT G (1), RESCH H (1), SCHMETTERER L (1, 3)*
*(1) Department of Clinical Pharmacology, Vienna*
*(2) Department of Ophthalmology, Vienna*
*(3) Center of Biomedical Engineering and Physics, Vienna*

**Purpose** It has been shown that increased neural activity evoked by stimulation with diffuse luminance flicker increases retinal and optic nerve head blood flow. Beside others, an increased oxygen demand has been attributed to evoke the flicker response. Thus, this study seeks to investigate whether the flicker light induced increase in retinal vessel diameters is altered under elevated blood pO2.

**Methods** 10 healthy volunteers were included in the study. Diameters of retinal vessels were recorded continuously with a Retinal Vessel Analyzer. During this measurement flicker stimulation was applied at a frequency of 8 Hz. Flicker responses were assessed before and during breathing of 100% oxygen. Blood gas values were determined from capillary blood samples.

**Results** Under room air conditions flicker stimulation increased retinal vessel diameters by +2.0±1.2% in retinal arteries and by +1.4±0.8% in retinal veins. Breathing of oxygen increased pO2 from 85±5 mmHg to 390±80 mmHg. Arterial diameter decreased by −9±8%, retinal venous diameters by −15±6% during the breathing period ($p<0.01$ each). No significant change in flicker response was observed during breathing oxygen compared to baseline conditions (arteries: 2.1±2.0%, veins: 1.1±1.0%).

**Conclusion** Breathing of oxygen does not alter the response of retinal vessels to stimulation with flicker light. This contradicts the hypothesis that increased oxygen demand is responsible for the flicker induced increase in vessel diameters.

## ▪ 2234
## Local retinal venous reaction to monochromatic flickering light in normal volunteers of different age

*KOTLIAR KE (1), VILSER W (2), MÜCKE B (1), LANZL IM (1)*
*(1) Ophthalmology, Munich University of Technology, Munich*
*(2) Biomedical Engineering, Ilmenau University of Technology, Ilmenau*

**Purpose** Retinal vessels and their reactions to stimuli are accessible to non-invasive imaging. Retinal vessels react to flicker stimulation. Differences in local venous reaction to flicker in healthy persons of different age are assessed.

**Methods** 3 groups of 11 healthy volunteers of 20-30 years, 40-60 years and 60-85 years each were examined by retinal vessel analyzer (RVA). After baseline assessment for 1 min a monochromatic (530-600nm) rectangular luminance flicker of 12,5 Hz was applied for 60 s. Diameters of venous segments were measured. Differences in amplitude and frequency of vessel widths change was characterized by the parameter 'spectral edge frequency' (SEF) before, during and after stimulation.

**Results** SEF in reciprocal measuring units:.....20-30 years.....40-60 years.....61-85 yearsbaseline.....0,090 ± 0,027.....0,114 ± 0,040.....0,137 ± 0,041 *dilation.....0,108 ± 0,042.....0,123 ± 0,048.....0,147 ± 0,038 relaxation....0,120 ± 0,029.....0,114 ± 0,040.....0,124 ± 0,044 We found a statistically significant difference in SEF between the youngest and the oldest age group but only at baseline ($p<0,05$, U-test).

**Conclusion** Retinal branch vessels possess alternating vessel diameters which constitute the vessel cross-sectional profile, assessable by RVA. We describe the parameter SEF, which shows presence of high frequent waviness of the vessel wall in its longitudinal cross-section. Previously we found in arteries a significant difference of this parameter in both older groups compared to younger volunteers in each phase except baseline. It seems, that veins do not posses as pronounced functional age-related structural changes as arteries.

■ 2235

## Effects of latanoprost and timolol on oxygen saturation in retinal vessels in healthy humans

*BALEANU D, LÄMMER R, SCIBOR M, HARAZNY J, MICHELSON G*
*Ophthalmology, Erlangen*

**Purpose** To determine the effects of topical application of latanoprost (LP) and timolol (TO) on oxygen saturation in retinal vessels.

**Methods** 40 healthy subjects (18-40 yrs) received 1 drop of TO or LP in 1 eye and 1 drop of placebo in the same eye for 5 days each. It was evaluated the oxygen saturation (OSart & OSven) in retinal vessels at baseline and after 5 days of treatment (tx) by using the Imaging Spectrometer. In 24 subjects the retinal capillary perfusion (flow) was measured by Scanning Laser Doppler Flowmetry (HRF). The arterio-venous difference (avD) was calculated. The product of flow and avD, as measure for the oxygen release into the ocular tissue, was determined: OxyR = flow x avD.

**Results** No significant changes in the OSven, significant decrease of OSart and of the avD in both tx groups. Flow- constant in the LP group and tendence of decrease (n.s.) in the TO group. Mean OxyR - no change in the LP group, but significant decrease in the TO group- mean relative change to baseline: LP -3,6% (n.s.), TO -14,7%*($p<0,05$)

**Conclusion** Topical application of both, LP and TO, resulted in a small decrease of OSart. A possible reason could be an increased vessel walls' permeability, that would permit an increased oxygen diffusion from the central retinal artery into the optic nerve. Tx by LP did not influence significantly the OxyR into the ocular tissue, whereas tx by TO resulted in a mean decrease of the OxyR into the tissue of 14,7%. This might be due to a compensatory mechanism regulating the decrease in the avD by individual adaptation of the retinal flow in the LP group, whereas this mechanism might fail in the TO group because of the ß-adrenergic effect with additional lowering of the retinal blood flow.

## ▪ 2252
## Experimental uveitis : introducing a new model

*TERRADA C (1), SALOMON BL (1), BODAGHI B (2)*
*(1) UMR 7087 CNRS/UPMC, Paris*
*(2) Ophthalmology, Paris*

**Purpose** The eye is an immune privileged site which suffers from Th1 type responses that induce severe uveitis. In many clinical cases, uveitis develops secondary to an ocular infectious disease. This could result from peripheral activation, followed by ocular penetration of T cells specific for a foreign antigen expressed in the retina. To test this hypothesis, we developed a new model of experimental uveitis

**Methods** One month after expression of the non-self neoantigen influenza virus hemagglutinin (HA) in the retina of BALB/c mice by gene transfer, uveitis was induced by adoptive transfer of HA-transgenic T cells, followed by subcutaneous HA immunization. Clinical examination of animals was performed with a slit lamp biomicroscope. Infiltration of donor cells was detected by immunostaining on retina flatmounts with anti-Thy-1.1 antibody and was studied using FACS analysis.

**Results** Intraocular inflammation was clinically and histologically detected in all animals, between 10 to 15 days after immunization with HA. Chorioretinal infiltrates and lesions were identified after histopathological analysis. Intraocular inflammation was clinically and histologically detected in all animals within 15 days. The ocular infiltrate was composed mostly of macrophages and HA-specific T-cells with a Th1 cytokine profile. Depletion of CD25+ T-cells exacerbated the disease, indicating their regulatory function in uveitis.

**Conclusion** This new animal model validates a non-autoimmune mechanism of uveitis, mimicking uveitis due to ocular infection. This model should be useful for tracking and studying pathogenic and regulatory T cells and testing new therapeutic options.

## ▪ 2253
## New concepts in the classification of choroiditis

*HERBORT CP (1, 2)*
*(1) Inflammatory Eye Diseases, La Source Eye Centre, Lausanne*
*(2) University of Lausanne, Lausanne*

**Purpose** Since the availability of indocyanine green angiography (ICGA), more detailed investigation of the choroid was made possible, giving information on early and/or subclinical disease, on the structures involved by the inflammatory process leading to a more appropriate classification based on the mechanisms of choroidal inflammation. Some of these mechanisms have been verified histopathologically while others are still presumed and need manifest proofs.

**Methods** A standard ICGA protocol to analyse choroiditis has been used. The angioraphic procedure comprises 3 main phases; the early phase up to 2-3 minutes showing superimposed retinal and choroidal large vessels and incipient exudation of the dye through the choriocapillaris into the choroidal stroma. The intermediate phase at about 10 minutes shows maximum choroidal stromal background fluorescence and the late phase at about 32 minutes shows wash-out of the dye from the general circulation with the large choroidal vessels appearing dark against the background stromal fluorescence.

**Results** ICGA has allowed to reclassify choroidal inflammation according to the structure that is preponderantly or initially involved. At the present stage of our knowledge there seem to be at least two main mechanisms of inflammation touching the choroid, diseases situated at the level of the choriocapillaris called primary inflammatory choriocapillaropathies that include most of the former white dot syndromes and diseases situated in the stromal portion of the choroid called stromal choroiditis of which Vogt-Koyanag-Harada disease is a typical example.

**Conclusion** Thanks to the imaging access to the choroid gained through ICGA different inflammatory mechanisms involving the choroid could be sensed, leading to a more adequate classification of choroiditis.

## ▪ 2254
## Introducing the new concept of “ocular vasculitis”

*ABU EL ASRAR AM*
*Department of Ophthalmology, College of Medicine, King Saud University, Riyadh*

**Purpose** In addition to retinal vasculitis, ocular vasculitis can present in many other forms, including episcleritis, scleritis, peripheral ulcerative keratitis, choroidal vasculitis, optic nerve vasculitis, and orbital and adnexal lesions. It may occur as an isolated ocular condition, as a manifestation of infectious or neoplastic disorders, or in association with a systemic inflammatory disease. Prompt diagnosis and institution of the appropriate therapy will help to control the ocular disease and, more importantly, the systemic disease. Thus it is essential that a thorough history, review of systems, and physical examination are performed in patients with ocular vasculitis. A multidisciplinary team approach is essential in the evaluation and treatment of these patients. The diagnostic evaluation should be focused, guided by the information obtained in the patient's history, and in their ophthalmic and physical examination.

## ▪ 2255
## Immunopathology of intraocular inflammation and evolving concepts in immunotherapy

*DICK AD*
*Department of Clinical Sciences, University of Bristol, Bristol*

**Purpose** Generally it is accepted that immune processes involved in posterior segment intraocular inflammation are largely antigen-specific CD4+ T cell driven. Not surprisingly however, at the time of clinical disease, the pathology observed in both man and in the plethora of animal models show that destruction is largely secondary to non-specific inflammation from bystander leukocytes and loss of tissue control of inflammation. Therefore effective therapy can be targeted to: (i) suppress acute inflammation to induce drug-remission (ii) redress immunological tolerance to maintain drug free remission and (iii) restore tissue homeostasis to also maintain remission and provide possibilities of regeneration. We will discuss immune targets with respect to pathology, where currently with the advent of 'biologics' and the possibilities of 'gene therapy' and cell based therapies together may achieve the above aims.

■ **2256**

## How OCT is changing our appraisal of inflammatory retinal diseases

*DE SMET MD, VAN VELTHOVEN MEJ, VERBRAAK FD*
*Ophthalmology, University of Amsterdam, Amsterdam*

**Purpose** Demonstrate the use of optical coherence tomography in the diagnosis and management of patients with ocular inflammation.

**Methods** The Zeiss Stratus OCT and the OCT Ophthalmoscope from OTI were used in the assessment of patients with uveitis. In most patients, additonal imaging modalities were used. Scans were repeated on subsequent visits. Scans were assessed for structural changes (retinal thickness, edema, atrophy, pigment alterations), clarity and integrity of scans. Correlation with other imaging techniques was made wherever possible.

**Results** OCT provides a unique insight in location, severity, and extent of active inflammation (eg location of retinal haze in Serpiginous/APMPPE versus Multifocal Choroiditis), or the degree of vascular incompetence (eg Birdshot retinochoroiditis or VKH). Assessing the degree of response to treatment, for example the resolution of macular edema is also facilitated. However, care must be made in interpreting the OCT findings in the light of known pathophysiologic mechanisms, and limitations related to imaging modality being used. It is also important to correlated OCT fndings with clinical findings, as the quality of scan is dependent on the training level of the technician performing the scan.

**Conclusion** OCT is a valuable tool in the assessment of patients with active inflammation and in determining the severity of complications or response to treatment. Being less invasive than angiography, it can easily be repeated allowing for a careful monitoring of response.

## ▪ 2261
## Binocular vison changes in young adults during a period of 3 years

*JORGE J (1), FERNANDES P (1), ALMEIDA J B (2), PARAFITA M A (3)*
*(1) Physics (Optometry), Braga*
*(2) Physics, Braga*
*(3) Surgery (Ophthalmology), Santiago Compostela*

**Purpose** This study assessed the phoric and vergences changes during a period of 3 years in a group of universitary students.

**Methods** In a longitudinal study 118 universitary students (34 males and 84 females) with a mean age 20.5 ± 2.33 years (mean ± SD), received two visual examinations at intervals ranging from 30 to 38 months. The examinations consist in the subjective refraction with and without cycloplegic, dissociated phoria and vergences in distant (DV) and near vision (NV) in the phoropter.

**Results** For the phoria state (1st/2nd visit) we found (0.43 exo / 0.88 exo) in DV and (0.42 eso/ 1.16 exo) in NV.For the vergences (blur / diplopia / recovery) at DV we found for the base-in (BI) (7.9/13.0/5.2) on the 1st visit and (8.9/10.7/6.0) on the 2nd, for the base-out (BO) the results was (13.0/23.8/8.0) on the 1st visit and (12.0/22.0/10.6) on the 2nd. In NV the results for the BI vergences at DV was (15.8/22.9/11.5) on the 1st visit and (13.9/19.6/11.8) on the 2nd. And for the BO vergences the results was (19.7/29.7/13.7) on the 1st visit and (20.6/28.0/15.9) on the 2nd. All the results are expressed in prismatic diopters. For the near point of convergence we found (Diplopia/Recovery) (2.6 /12.9 cm) on the 1st visit and (2.9 /12.4 cm) on the 2nd.We found statistically significant differences ($p < 0.05$) for the phoria DV and NV. For the BI vergences we also found statically significant differences for diplopia and recover in distant vision and for blur and diplopia in near vision. For the BO vergences, the differences for the diplopia and recovery in distant and near vision are also statically significant.

**Conclusion** The binocular vision shows a great instability with significantly alterations in the phoria and vergences states.

## ▪ 2262
## Distance Stereoacuity in Intermittent Exotropia

*HOLMES JM*
*Mayo Clinic College of Medicine, Rochester, MN*

**Purpose** Last year at EVER 2004, we presented preliminary data on the utility of the new Frisby Davis 2 (FD2) distance stereotest in constant versus intermittent exotropia (IXT). Distance stereoacuity has been proposed as a means to monitor for IXT deterioration, therefore we evaluated new tests of distance stereoacuity across a range of IXT severity.

**Methods** Nine patients with IXT were prospectively enrolled with intermittent distance exotropia ranging from 3 pd to 70 pd, and a distance control score from 1 to 4, on a scale of 0 to 5, where 5 is always tropic. Distance stereoacuity was evaluated with the FD2 test and a newly developed Distance Randot test. Correlations were evaluated with Spearman rank correlation coefficients (rs).

**Results** Overall, FD2 scores were excellent (range 20 to 160 secarc, median 40 secarc) but were poorly correlated with distance control scores (rs=0.12). Patients with poor control (grade 4) could demonstrate excellent FD2 stereoacuity. The New Distance Randot stereotest showed a greater range of scores in these patients (60 secarc to nil, median 200 secarc), and modest correlation with distance control scores (rs=0.49), but there were notable examples of patients with excellent control and no Distance Randot stereoacuity. Correlations with angle of deviation were only modest (rs=0.44 and 0.48).

**Conclusion** Patients with large angle IXT and poor distance control may still demonstrate excellent distance stereoacuity by FD2, limiting the test's usefulness in monitoring deterioration of IXT. The new Distance Randot test shows more promise in identifying IXT patients with poor control, but further modification of the test is needed to address failure by occasional IXT patients with excellent control and near stereoacuity.

## ▪ 2263
## Thomsen myotonia congenita and strabismus, saccades recording in myotonic diseases

*SPIRE M (1), HABAULT C (2), TILIKETE C (3), VIAL JC (4), GRANGE JD (1)*
*(1) Ophtalmology Croix-Rousse Hospital, Lyon*
*(2) Ophtalmology, Lyon*
*(3) Ophtalmology and neuro-ophtalmology Wertheimer Hospital, Lyon*
*(4) Neurology and fonctional exploration Wertheimer Hospital, Lyon*

**Purpose** To report an original family case of Thomsen myotonia disorder associated with strabismus and saccades alterations traducing an ocular myopathy.

**Methods** Thomsen myotonia is an autosomal dominant, no dystrophic myotonia. Four women of the same family presented a Thomsen myotonia and strabismus. Their electro-oculographic analysis shows saccades alterations. We studied also 7 isolated patients presenting a myotonic disease and 2 witnesses; we were interested in the recording of the ocular saccades of these patients as a comparison for our family case. The saccades of the seven isolated patients have been evaluated by electro-oculographic recording, according 4 different paradigms.

**Results** The patients, within our family case, presented an esotropia, with or without a vertical oculo-motor disorder. Clinical examination reported alterations of ocular motility suggesting a myopathic disorder. The electro-oculographic recordings reported a slowing of saccades with increasing duration. For the isolated cases, the slowing of saccades was only reported for the patients with myotonic dystrophy (Steinert), but we failed in showing any myotonic phenomenon. In literature: Thomsen myotonia, strabismus and electro-oculographic disorders have never been reported before. A revue of literature didn't find a similar association. The electro-oculographic slowing down of saccades is not rare in myotonic dystrophy but in Thomsen myotonia this constatation has never been reported.

**Conclusion** It is the first original report concerning Thomsen myotonia associated with strabismus and alterations of saccades. The mechanism of this disorder has still to be discovered, as for Steinert disease.

## ▪ 2264
## Eyelid and eye movements in Bell's palsy

*VAN DER WERF F (1), SMIT A (1), METSELAAR M (2)*
*(1) Neurosciences, Erasmus MC, Rotterdam*
*(2) Rhino-Otolaryngology, Erasmus MC, Rotterdam*

**Purpose** In a longitudinal study, recovery of bilateral eyelid and eye movements during blinking was investigated in patients with Bell's palsy. Kinematics of eyelid and eye movements and concomitant orbicularis oculi activity (OO-EMG) during various types of blinking were examined.

**Methods** Ten patients with Bell's palsy, which scored Brackmann House grade 5 and/or 6, were implemented in the study. Using the magnetic search coil technique and OO-EMG, eyelid movements were measured every 6 weeks; every 18 weeks eyelid and eye movements were recorded simultaneously.

**Results** The first OO-EMG activity was determined in the fourth month, 8 months later OO-EMG at the affected side showed similar values as at the healthy side. Eyelid kinematics during voluntary blinking had been recovered after twelve months, although their amplitudes were retarded. Their accompanied eye movements remained significantly aberrant till eighteen months and even sometimes longer. The affected eye movement is laterally and nasally upward instead of nasolateral downward. The healthy eye movement always follows eye movement at the affected side. Eyelid kinematics during reflex blinking was recovered after twelve months; their concomitant eye movements are normal although their amplitudes remain retarded at both sides.

**Conclusion** Modulation of basal ganglia in the supra-nuclear control of voluntary blinking remains mostly disturbed during this longitudinal study. Eyelid and eye characteristics during reflex blinking are normal 12 months after affection. Kinematics of eyelid and eye movements during reflex blinking raise to normal values, although amplitudes and maximal downward velocities of lid and eye movements remain smaller. These data indicate that Bell's palsy is a multifocal neuropathology.

■ **2265**

## A case of cerebral achromatopsia

*OEHRENS AM (1), BOSCHI A (2), DUPREZ T (3), IVANOIU A (4)*
*(1) Dept. of Ophthalmology, University Hospitals, KUL, Leuven*
*(2) Dept. of Ophthalmology, University Hospital, UCL, Brussels*
*(3) Dept. of Neuroradiology, University Hospital, UCL, Brussels*
*(4) Dept. of Neurology, University Hospital, UCL, Brussels*

**Purpose** Cerebral achromatopsia is an uncommon defect of color perception caused by damage to the visual cortex. The most common setting is vertebrobasilar ischemia affecting the blood supply to the occipital lobes. We report a case of cerebral achromatopsia combined with hominous hemianopsia, evolving during recovery of cortical blindness secondary to acute hydrocephalus in a young patient.

**Methods** Clinical history, neuro-ophtalmological signs , neuropsychological evaluation and MRI images are reported and correlated.

**Results** An 18 year-old student with a ventriculo-peritoneal drain for aqueduct stenosis since 2 months, developed an acute hydrocephalous complicated by a brainstem herniation and subsequent vertebrobasilar ischemia with bilateral vascular insufficiency in both occipital lobes. During recovery of cortical blindness, a right homonimous hemianopsia with full-field dyschromatopsia and visual agnosia and alexia were observed. Psychological and neurological evaluation showed severe visual and topographic memory deficit. Cerebral MRI showed a massif infart in the left occipito-temporal lobe and a little infarct of the right lingual occipito-temporal region. A follow up of the neuro-ophtalmological signs and radiological image is reported.

**Conclusion** These findings confirm that the ventromedial sectors of the occipital lobe in the lingual and fusiform gyri is important in the transmission of color visual information. Detection of complex visual dysfunction is a challenge in ophthalmology.

■ **2266**

## Venous retinal vessel response of migraine patients to luminant chromatic and monochromatic flicker application

*LANZL IM (1), WIESCHER A (1), VILSER W (2), KOTLIAR KE (1)*
*(1) Ophthalmology, Munich University of Technology, Munich*
*(2) Biomedical Engineering, Ilmenau University of Technology, Ilmenau*

**Purpose** Exposing the retina to flickering light is followed by retinal vessel dilation. Vessel responses could be different in different diseases.

**Methods** In 21 migraine patients (age 21-64 years) diameters of a retinal venous segment were examined by Retinal Vessel Analyzer (RVA). After 1,5 min of baseline a red/green and blue/green flicker light of 2, 12, or 20 Hz or monochromatic green flicker of 12,5 Hz was applied for 30 s. Subgroups of 10 patients each with the strongest and weakest manifestation of a migraine attribute were formed. The results (area under the vessel reaction curve during the stimulation in s*%) were compared using non-parametric statistics.

**Results** Average venous reaction of the whole group does not differ significantly from a healthy group. The patients were divided into subgroups. No significant difference for the characteristic "first attack, years ago"; "most recent attack, days ago" was found. For characteristics "duration of an attack" (4,7±2,9 / 52,8±34,8 h) with 6,4±27,9 and 61,6±34,9 ($p<0,01$); "often/rare attack appearance" (6,9±3,2 / 1,5±1,1 a month) with 54,2±44,5 and 10,3±34,3 ($p<0,04$); as well as for "aura yes/no" with 49,9±41,7 and 3,1±35,5 ($p<0,03$) a significant difference of the flicker response between the corresponding subgroups was found for blue/green flicker of 2 Hz (normal group: 65,3±20,2).

**Conclusion** In the subgroups formed according to migraine specific attributes for patients with a short duration and rare appearance of migraine attack the venous response was smaller and came temporally delayed. The patients with more severe migraines might develop some adaptation mechanisms, which could normalize their vessel reactions.

■ **2267 / 277**

## Two year's training of homonymous hemianopia with flicker stimulation

*RANINEN AN (1, 2, 3), HENRIKSSON L (4, 5, 6), VANNI S (4, 5, 6), HYVÄRINEN LVM (7), NÄSÄNEN R (8)*
*(1) University of Helsinki, Helsinki*
*(2) Bio and Environmental Sciences, Helsinki*
*(3) Physiology, Helsinki*
*(4) Helsinki University of Technology, Espoo*
*(5) Low Temperature Laboratory, Espoo*
*(6) Brain Research Unit, Espoo*
*(7) Lea-Test Ltd, Helsinki*
*(8) Finnish Institute of Occupational Health, Helsinki*

**Purpose** As a result of one-year training, the flicker sensitivity on the hemianopic side became equal to that of the intact side up to an eccentricity of 20 deg. Now, we wanted to find out, if continued training could further extend the area of equal sensitivity to even larger eccentricities. Hence, both men continued training for another year.

**Methods** Subjects KS and IT participated in two weekly training sessions, where a flickering disk was detected at eccentricities 30 (KS), 40 and 60 degrees (IT) along the horizontal meridian. Flicker frequencies were 1, 2, 5, 10, 15, 20, and 35 Hz. Neuromagnetic responses were followed during rehabilitation, and IT's fMRI activation to blind field stimulation was recorded after rehabilitation.

**Results** After 17 months of training the flicker sensitivities of subject IT became symmetric at the eccentricity of 40 deg. After an additional 5 months of training his flicker sensitivities in the hemianopic visual field at the eccentricity of 60 deg became nearly equal to that of the intact visual field. The only exception was the lower right quadrant, where sensitivities were lower than those of the intact side. In the end of the 2nd year of training flicker sensitivities of KS at 30 deg were symmetric. During training both IT's and KS's MEG recordings showed new evoked responses to hemianopic visual field stimulation. IT's fMRI showed that the two visual fields were represented in one set of retinotopic areas.

**Conclusion** Our findings prove that there is still plenty of plasticity in the brain at the age over 60-years. This work shows also that field loss in homonymous hemianopia can be rehabilitated with long-term flicker stimulation of the blind hemifield.

## ▪ 2311
## Stiles-Crawford effect

*BERENDSCHOT TTJM*
*University Eye Clinic Maastricht, Maastricht*

**Purpose** Light that enters the eye near the middle of the pupil is perceived to be brighter than light entering the eye near the pupil edge. This phenomenon, called the Stiles-Crawford effect after its inventors, is due to directionally dependent light absorption. It can also be observed in light reflected at the fundus, the so-called optical Stiles-Crawford effect. Setups acquiring the reflectance at different points across the (dilated) pupil that were developed to measure the Stiles-Crawford effect will be discussed. Determination of the Stiles-Crawford effect can be used to reveal changes in the dimensions or composition of the photoreceptors. This will be demonstrated by several examples.

## ▪ 2312
## Retinal imaging with Multiplanar OCT

*ROSEN R*

**ABSTRACT NOT PROVIDED**

## ▪ 2313
## Optophysiology: Non-invasive, depth resolved optical probing of retinal physiology

*DREXLER W, BIZHEVA K, PFLUG R, POVAZAY B, HERMANN B, SATTMANN H, QUI P, ANGER EM, REITSAMER HAR, POPOV S, TAYLOR JR, AHNELT PK*
*Center for Biomedical Engineering and Phyiscs, Vienna*

**Purpose** A functional extension of ultrahigh resolution OCT (UHR OCT) has been developed, that has the potential to establish this technique as an optical analogue to electrophysiology, by detecting depth resolved variations in optical backscattering caused by physiological tissue changes.

**Methods** Studies on excised, but physiologically intact, white light stimulated guinea pig and rabbit retinas have been performed. UHR OCT using a state of the art ultrabroad bandwidth light source centred around 1250 nm enabling sub-3 µm axial resolution has been synchronized with the light stimulus to properly detected spatially resolved alterations in optical backscattering over time caused by light-induced intraretinal, physiological changes. These optical signals were resolved with a time resolution less than 5 ms and have been correlated with simultaneous electroretinograms (ERG recordings).

**Results** Preliminary results demonstrate the potential of this novel extension of UHR OCT to detect time-dependent optical backscattering changes after application of single and multiple light stimuli in specific retinal layers, e.g. the inner and outer segments of the photoreceptor layer or the inner plexiform layer. Control experiments, including no light stimulus or application of drugs that could inhibit the physiological responses of certain type of retinal cells confirm the physiological origin of the detected backscattering changes.

**Conclusion** Detection of cell activity and cell physiology by UHR OCT would enable a better understanding of basic physiological phenomena and may also contribute to better understanding of retinal pathogenesis.

## ▪ 2314
## Hyperspectral imaging of the retina: principles and applications

*NASCIMENTO SMC*
*Department of Physics University of Minho, Braga*

**Purpose** Hyperspectral imaging is the acquisition of spatially resolved images in several spectrally narrow and almost adjacent wavelength bands. Two applications of the technique will be considered: mapping of spatial changes in the haemoglobin spectrum and estimates of the spatial extent of the macular pigment.

**Methods** A hyperspectral fundus imaging system was adapted from an indirect ophthalmoscope and the retinas of healthy subjects analyzed. An area of the retina of about 15 deg was illuminated by light from a xenon lamp filtered by a fast tunable liquid-crystal filter with bandwidth about 10 nm. Spectral images of the fundus were acquired sequentially over the visible spectral range with a low-noise Peltier-cooled digital camera with a spatial resolution of 1344 x 1024 pixels and 12-bit output. The relative reflectance spectrum of each individual pixel element was estimated against a standard white reference and the double optical density of the macular pigment as a function of the location in the retina was obtained.

**Results** Sampling from very small retinal areas allowed the spectral characterization of very fine details and, in particular, the observation of changes in oxygenation. The spatial distribution of macular pigment showed a large inter-subject variability, but a tendency for larger distributions with age and significant spatial asymmetries were found.

**Conclusion** Hyperspectral imaging may be a useful technique for fundus analysis in the normal and abnormal retinal conditions.

■ **2315**

## Functional and morphological retinal data fusion

*BERNARDES R*
*Center New Technologies for Medicine, Coimbra*

**Purpose** The aim of this work is to present the registration of two different and complementary imaging modalities of the human eye fundus.

**Methods** A digital color fundus photograph and a functional map of the human blood-retinal barrier, covering respectively the central 50 and 20 degrees FOV, will be registered using a deformable image registration process, after demonstrating the inacuracy of both the rigid and perspective image registration.

**Results** The registration of different modalities is achieved correcting for intrinsic image deformations due to the scanning process and using morphological and functional information.

**Conclusion** The possibility of registering different and complementary information, allows to achieve a multimodal image of the human eye fundus that integrates a larger set of information sources.

## ▪ 2321
## Dry eye related changes in the anterior eye segment

*DARTT DA (1, 2)*
*(1) Schepens Eye Research, Boston, MA*
*(2) Department of Ophthalmology, Harvard Medical School, Boston, MA*

**Purpose** The tear film protects the eye from the external environment. For this protection, tear secretion occurs rapidly in response to external stimuli.

**Methods** This response is provided by neural stimulation of lacrimal gland secretion, a major force driving tear production. Activation of corneal sensory nerves stimulates parasympathetic and sympathetic nerves innervating the lacrimal gland. Once activated, parasympathetic nerves release acetylcholine (ACh) and VIP, whereas sympathetic nerves release norepinephrine. Each neurotransmitter interacts with specific receptors, muscarinic ACh receptors (M3AChR), VIP receptor 1 and 2, and alpha1D-adrenergic receptors (a1D-ARs) for ACh, VIP, and norepinephrine, respectively and activates a separate, signaling pathway. Activation of M3AChRs increases intracellular Ca2+ and activates protein kinase C (PKC) isoforms to stimulate secretion. ACh activates the EGFR signaling pathway, distal to the EGFR, to stimulate p44/p42 mitogen-activated protein kinase (MAPK) activity negatively modulating secretion. Stimulation of a1D-ARs activates PKCe and produces nitric oxide (NO) and cGMP to induce secretion. Activation of a1D-ARs induces a matrix metalloproteinase to cause ectodomain shedding of EGF. Shed EGF stimulates the EGFR to activate MAPK negatively modulating secretion.

**Results** Disruption of neural regulation of lacrimal gland secretion occurs in LASIK surgery, aging, and Sjogren's syndrome leading to aqueous deficiency dry eye. In each of these conditions neurally activated signaling pathways are disrupted at distinct sites leading to decreased lacrimal gland secretion and aqueous deficiency dry eye.

**Conclusion** Findings provide the basis for developing novel treatments for dry eye syndromes.

## ▪ 2322
## In vivo confocal microscopy of dry eye patients with SS and non-SS

*BENITEZ DE CASTILLO JM (1, 2)*
*(1) Hospital Clinico San Carlos, Unidad de Superficie e Inflamacion Ocular, Madrid*
*(2) Universidad Complutense, Madrid*

**Purpose** The ocular surface, the main lacrimal gland and the interconnecting neural reflex loops comprise a functional unit. An alteration in corneal innervation has been described in dry eye (DE) associated with diabetes mellitus, contact lens use and lasik. The purpose of this study was to evaluate if DE not related with Sjogren´s syndrome (NSDE) and DE related to this syndrome (SSDE) are associated with an alteration of the corneal nerves.

**Methods** Group healthy volunteers less 60 yo (HVl60)(10 females and 1 male) aged 30.7 SD 2.6, group healthy volunteers more 60 yo (HVm60)(8 females and 2 males) aged 68.7 SD 7.1, group SSDE (10 females and 1 male) aged 52.9 SD 8.7 and group NSDE (8 females and 2 males) aged 65.8 SD 5.3 yo. Patients were examined with a Confoscan model P4 (Tomey, Germany).

**Results** Sub-basal nerve density: HVl60 = 769 SD 88, HVm60 = 624 SD 86, SSDE = 508 SD 128 and NSDE = 593 SD 127 microns/mm2 ($p<0.000$, ANOVA). There was a statistically significant difference between HVl60 and the other groups (HVm60 $p<0.05$, SSDE $p<0.000$ and NSDE $p<0.005$, Bonferroni). Number of beadings: HVl60 = 198 SD 66, HVm60 = 182 SD 63, SSDE = 387 SD 62 and NSDE = 323 SD 64 /mm ($p<0.000$, ANOVA). There was a statistically significant difference between healthy volunteers groups and DE groups ($p<0.000$, Bonferroni).

**Conclusion** SSDE and NSDE patients exhibit corneal innervation abnormalities.

## ▪ 2323
## Ultrastuctural changes of the corneal nerves after LASIK

*MÜLLER LJ*
*Ocular Signal Transduction, Amsterdam*

**Purpose** LASIK is preferred over PRK because the patient does not suffer from pain and vision is almost immediately restored. As the microtome-cut disconnects many stromal from epithelial nerves it is essential to analyse the corneal nerves after unsuccessful LASIK-treatment.

**Methods** Two LASIK patients 32 and 56 yrs old, underwent 2 yrs post-op corneal transplantation: 1) due to ectasia) and 2) due to problems related to epithelial ingrowth. Both corneal buttons were immediately fixed in the operating theatre after surgery and analysed in detail with light and electron microscopy.

**Results** In one cornea the inner part of the flap was covered with epithelial cells and in the other one the interface of the flap was located in the mid stroma at approximately 250 µm. Nerve terminals within the flap were normal, whereas those at the surface of both corneas were drastically affected. The cornea with ectasia, had beautiful nerves in the posterior stroma at locations where stromal nerves are usually not found. These observations will be compared with recent data on the organization of human corneal nerves.

**Conclusion** This is one of the scarce studies showing the ultrastructure of nerves after LASIK. Although both corneal buttons were removed due to failure the few stromal nerves present were well preserved. Whether they are newly formed or survived after LASIK is unknown.

## ▪ 2324
## Is there a difference in corneal nerves in dry eye patients and patients after refractive surgery?

*TUISKU IS, TERVO TM*
*Helsinki University Eye Hospital, Helsinki*

**Purpose** To review the morphological and functional changes in corneal innervation in primary Sjögren´s syndrome (pSS) dry eye as well as during reinnervation process in patients undergone refractive surgery.

**Methods** The modified Belmonte non-contact esthesiometer and in vivo confocal microscopy has been used.

**Results** Dry eye symptoms after LASIK have been attributed to nerve damage during surgery. Nerve regeneration after LASIK is relatively slow process. Eventually, two years after LASIK corneas show normal subbasal nerve density. After surgery an initial hypersensitivity was seen, but subsequently LASIK corneas showed decreased sensitivity. Eventually, at five years after LASIK, sensitivity treshold values did not differ from controls. Interestingly, even after five years the majority of patients reported dry eye symptoms, although objective clinical signs of dry eye were not demonstrable.Patients with pSS the subbasal nerve density was similar to controls. Interestingly, morphological nerve alterations (nerve growth cone-like patterns and/or Langerhans cells) were seen. Corneal sensitivity threshold values were significantly lower in pSS compared to controls, implying corneal hypersensitivity.

**Conclusion** Refractive surgery induces a long-lasting disturbance in corneal innervation. Subjective discomfort did not correlate with tear flow tests in LASIK patients operated 5 years earlier. This might suggest that sensitization of the nerves or abnormal regeneration might be involved. In pSS, inflammatory changes among subbasal nerves may contribute to hypersensitivity seen in these patients. This may clarify clinical paradox often seen between symptoms and signs in these patients.

■ 2325

## Possible approaches to treat patients with dry eyes

*KRUSE FE*
*Dept of Ophthalmology, Univ. of Erlangen, Erlangen*

ABSTRACT NOT PROVIDED

■ 2331

## VEGF concentration in vitreous from diabetic patients is not associated with hmg-coa reductase inhibitor (statin) therapy

*LIINAMAA MJ (1, 2), SAVOLAINEN MJ (2)*
*(1) Dept. of Ophthalmology, University of Oulu, Oulu*
*(2) Dept. of Internal Medicine and Clinical Research Center, University of Oulu, Oulu*

**Purpose** Vascular endothelial growth factor (VEGF) has an important role in the development of diabetic retinopathy. Previous studies have suggested that statin therapy may retard progression of diabetic retinopathy. In addition to the cholesterol-lowering effect of statins, they are known to improve endothelial function, and might therefore be protective in diabetic retinopathy by influencing vitreous VEGF concentration. The effect of statin therapy on the vitreous VEGF concentration was assessed in patients with proliferative diabetic retinopathy or diabetic maculopathy.

**Methods** The study population consisted of 55 diabetics (with type I or type II diabetes) and 43 non-diabetic subjects who underwent vitreous surgery. The study subjects were interviewed about the use of statin medication. Undiluted vitreous samples (200 to 500 µl) were collected at the beginning of vitrectomy. Vitreous VEGF concentrations were assessed using a chemiluminiscent human VEGF immunoassay.

**Results** The statin users (n=13) and the controls (n=32) did not differ significantly in concentration of vitreous VEGF, fasting blood glucose, glycated hemoglobin, blood pressure or in duration of diabetes. Furthermore, no significant changes in vitreous VEGF concentration were found in diabetic group when divided according to diabetes type or type of retinopathy.

**Conclusion** Current results show for the first time that statin therapy is not associated with changes in vitreous VEGF concentration. The potentially beneficial effects of statins suggested in previous clinical studies on diabetic retinopathy may not be mediated through a decrease in VEGF concentration in the eye.

■ 2332

## Antioxidant and angiogenic protein expression in diabetic retinopathy

*DE SOUZA RAMALHO P (1, 2), PEREIRA DA SILVA A (2), THIRAN C (2), FONTES G (2), BICHO MP (2)*
*(1) Ophtalmology, Lisbon*
*(2) Genetic Laboratory, Encrinology and Metabolism Center, Lisbon University Medical College, Lisbon*

**Purpose** Diabetic Retinopathy is a multifactorial process. There is growing evidence of inflammation, oxidative stress and genetic involvement in diabetic retinopathy. Transmembrane reductase (TMR) erithropoietin (EPO) and haptoglobin (Hp) have antioxidant properties besides other functions.ObjectiveTo verify the role of 3 antioxidant proteins in diabetic retinopathy.

**Methods** A sample of 58 diabetics mainly tipe II (95.1% tipe II, 4.9% tipe I), 24 with and 34 without retinopathy, mean age of 64.21 ± 11.64 years of both sexes and 44 controls age and sex adjusted was studied. TMR (mmol/cel/h) was determined by spectophotomethry; EPO (mIU/ml) by ELISA and Hp phenotypes using polyacrilamide gel electrophoresis. Statistical analysis was done by Student t test, ANOVA, $\chi$ 2 and Pearson correlation.

**Results** In retinopathy subjects there was higher TMR activity (5.29 ± 2.11 vs 4.11 ± 1.51 in controls, p=0.016), higher levels of EPO (15.15 ± 11.14 vs 10.08 ± 6.63 in controls, p=0.043) and predominance of Hp 2 2 genotype (40.9%). Hp 2 1 genotype has more incidence in diabetics without retinopathy (70.6%) p=0.028 similar to the controls. There was significant difference in EPO levels in diabetics (13.46 ± 11.45) compared to the controls (9.47 ± 6.57) p=0.043.

**Conclusion** Antioxidant activity mediated by TMR, EPO and Hp and angiogenic tendency linked with EPO and Hp 2 2 genotype seems to be associated with retinopathy process. These changes could be good predictable retinopathy markers indicating further therapeutic/pharmacologic intervention.

■ 2333

## Can erytropoietin protect retinal cell injury in diabetic retinopathy ?

*DE SOUZA RAMALHO P (1), PEREIRA DA SILVA A (2), THIRAN C (2), BICHO M (2)*
*(1) Ophthalmology, Genetic Lab, Lisbon University, Medical College, Lisbon*
*(2) Genetic Lab, Lisbon University, Lisbon*

**Purpose** Erytropoietin (EPO) a multifunctional cytokine (40.3 KD) consisting of 165 aminoacids, produced by interstitial kidney cells in adults and present in CNS, retina, heart and others, is expressed in the serum. It crosses blood brain/retinal barriers.EPO is upregulated in ischaemic retina and light induced retinal degeneration. Exogenous hrEPO administration can provide neuroprotective effects against cellular damage in diabetic rats. High concentration of EPO in proliferative retinopathy in diabetic vitreous was reported. Previous study suggested a tendency towards higher EPO serum expression in diabetic retinopathy.ObjectivesTo study in a larger sample serum EPO expression in diabetics with and without retinopathy.

**Methods** In 58 type II diabetics, 24 with and 34 without retinopathy of both sexes and mean age of 64.24 ± 11.64, EPO expression in serum was determined using ELISA (mIU/ml) and compared to 44 non diabetic controls age and sex matched. Student t test was done.

**Results** Serum EPO levels showed higher values in diabetics with retinopathy 15.15 ± 11.44, without retinopathy 10.084 ± 6.63, p=0.043. Controls 9.47 ± 6.57 mIU/ml.

**Conclusion** Increased expression of serum EPO in diabetics with retinopathy supports endogenous over production, an atempt for neuroprotective effect which was not achieved because of its low values. Exogenous EPO may be necessary to protect the retina from hypoxic injury. Constitutive EPO seems to be insufficient to give protection which is only achieved in some concentrations. Thus EPO could be an important target molecule for pharmacologic intervention in diabetic retinopathy. Clinical trials are necessary.

■ 2334

## Docosahexaenoic acid and ether-lipid changes and ERG alterations during retinal ischemia-reperfusion in the rat

*BRON AM (1), ACAR N (2), JOFFRE C (2), GREGOIRE S (2), ARNAVIELLE S (1), GARCHER CREUZOT CP (1), BRETILLON L (2)*
*(1) Ophthalmology, University Hospital, Dijon*
*(2) Eye and Nutrition Research Group, Dijon*

**Purpose** To evaluate the variations of retinal lipids known to be sensitive to oxidative stress -docosahexaenoic acid and ether lipids- after ischemia-reperfusion in rats. The relationships with retinal function were also studied.

**Methods** Retinal ischemia was induced on Wistar rats for 60 minutes by raising intraocular pressure to 110 mm Hg by canulation of the anterior chamber of one eye. The other eye was used as control. After 24 hours, 7 days and 14 days of reperfusion, the scotopic ERG was recorded on both eyes. Lipids were extracted from retinas and analyzed by gas chromatography.

**Results** The b-wave amplitude showed a partial time-dependent recovery as judged by the reduction observed in treated eyes compared to controls: -82%, -58%, -51% after 24 hours, 7 days and 14 days of reperfusion, respectively. By contrast, a stable 50%-reduction of the a-wave amplitude was observed in the ischemic eye. These alterations in the visual function were correlated to a significant decrease in retinal docosahexaenoic acid levels (-16% after 14 days of reperfusion). Within ether-lipids, species linked to oleic acid were significantly reduced in treated eyes after 14 days of reperfusion.

**Conclusion** This study strongly suggests a link between retinal lipid levels and retinal function, as illustrated by the ERG a-wave amplitude. Based on the modification of the ether-lipid content, our results provide new data on their putative anti-oxidative properties roles within the retina.

■ 2335

## Early Diabetes-Induced Changes in the Retina: Comparison of Rat and Mouse

*OBROSOVA IG (1), PACHER P (2), SEIGEL G (3), STEVENS MJ (4)*
*(1) Pennington Biomedical Research Center, Baton Rouge, LA*
*(2) NIH/NIAAA, Rockville, MD*
*(3) University at Buffalo, The State University of New York, Buffalo, NY*
*(4) University of Michigan, Ann Arbor, MI*

**Purpose** The purpose of this work was to compare early retinal changes in streptozotocin(STZ)-diabetic rat and mouse models.

**Methods** The experiments were performed on control (C) and diabetic (D, 6-wk duration) Wistar rats and C57Bl6/J mice. Retinal glucose, sorbitol, fructose, lactate, pyruvate, glutamate, alpha-ketoglutarate, and ammonia were assayed spectrofluorometrically by enzymatic procedures, VEGF by ELISA and poly(ADP-ribose) and apoptosis by immunohistochemistry. Free mitochondrial and cytosolic NAD+/NADH ratios were calculated from the dehydrogenase systems.

**Results** Retinal glucose levels were increased 6- and 5-fold in D rats and mice. D rats manifested ~10-fold and 6-fold sorbitol and fructose accumulation whereas the increase of both metabolites in D mice was modest (< 2-fold). D rats had increased malondialdehyde plus 4-hydroxyalkenal levels, reduced superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione transferase activities and increased poly(ADP-ribose) immunoreactivity whereas mice had no manifestations of retinal oxidative stress or poly(ADP-ribosyl)ation. D rats had 1-3 TUNEL positive cells in the neural retina per 4 microm thick eye cross-section whereas all D mouse samples were negative. D rats had decreased free mitochondrial and cytosolic NAD+/NADH ratios, consistent with retinal hypoxia, whereas D mice preserved both ratios in the normal range.

**Conclusion** In contrast to STZ-D rat, STZ-D mouse does not represent a good model for studying the roles for sorbitol pathway, oxidative stress, PARP, and NAD+/NADH redox changes in early DR. It remains to be established if the mouse model is suitable for exploring contribution of these mechanisms to later retinal changes in diabetes.

■ 2336

## Expression of EphrinB2 and EphB4 in a mouse model of oxygen-induced retinopathy (OIR)

*EHLKEN C, MARTIN G, HANSEN LL, AGOSTINI HT*
*University Eye Hospital, Freiburg*

**Purpose** The transmembrane ligand EphrinB2 and its membrane-bound receptor EphB4 are expressed on arterial and venous endothelial cells, respectively. Being expressed even at very early stages of vascular development, a role in angiogenesis is discussed. The purpose of this study was to examine the expression of EphrinB2, EphB4 and of vascular endothelial growth factor (VEGF) in a murine model of oxygen-induced retinal neovascularization.

**Methods** Mice were kept at 75% oxygen between postnatal days 7 (P7) and P12. The return to room air causes a relative hypoxia and mice develop retinal neovascularization within 5 days. At intervals, total retinal mRNA was isolated for measuring expression of EphrinB2, EphB4, VEGF and its receptors VEGFR1 and VEGFR2 by quantitative RT-PCR.

**Results** In the OIR model, VEGF expression is significantly increased after 12 hours of relative hypoxia, with a maximum factor of 2,8 at P16. While EphrinB2 was almost constantly expressed, EphB4, however, showed a tendency towards increased expression, but reached statistical significance only at P14. Under physiological conditions without oxygen treatment, there is no significant change of expression of either EphrinB2, EphB4 or VEGF.

**Conclusion** In comparison to VEGF, regulation of EphrinB2 and EphB4 gene expression seems to play a minor role in pathological retinal neovascularization. This does not exclude, however, activation of EphrinB2 and EphB4 on the protein level during angiogenesis.

■ 2351

## Immunomodulation of TH1 response a science overview...what are we trying to achieve?

*DICK AD*
*Department of Clinical Sciences, University of Bristol, Bristol*

**Purpose** Many intraocular inflammatory conditions which are well represented in animal models of uveitis are mediated by consequences of Th1 CD4+ T cell activation. Accordingly, the advent of 'biologic' therapy has increased our ability to specifically target immune modulators, including cytokines, chemokines, adhesion molecules as well as leukocytes themselves. However, it also remains apparent that the multiplicity of cell and cytokine function is dependent upon dose, chronicity and timing within the disease course and therefore predicting outcome may be challenging.

**Methods** Animal models have helped detail many immunologic mechanisms of disease expression during autoimmune responses; and with the use of gene altered mice we have been able to further probe for therapeutic targets. However, translation to man is not always as successful and it requires concomitant data to support biologic targeting in any given specific disease.

**Results** There has been successful preclinical work and translational studies showing benefit for IL-2R and TNF alpha blockade. However, more recent experimental evidence supports the targetting of IL-23 driven IL-17+ CD4+ T cells as the main mediators of autoimmune responses. Moreover immunomodulatory therapies that generate or restore T reg cells is of likely necessity to sustain remission induced by biologics. This may be achieved as supported by animal data via generating tolerance by mucosal peptide therapy or Dendritic cell based therapies.

**Conclusion** To date we have been successful in ameliorating inflammation but there is less evidence that immunomodulation occurs by either standard immunotherapy or via newer biologic therapies and moreover there is no evidence unlike animal models that we induce drug-free remission.

■ 2352

## TNF blockade in ocular inflammatory disease

*SMITH J*
*Casey Eye Institute, Oregon Health & Science University, Portland, OR, USA*

**Purpose** Tumor necrosis factor alpha (TNF-α) is an inflammatory cytokine secreted by diverse cell types in response to infectious and inflammatory stimuli. Its actions are signaled via 2 receptors, p55 or TNFR1, and p75 or TNFR2. Because TNF-α has been implicated as a key mediator of diverse inflammatory diseases, including various forms of ocular inflammation, it presents an ideal target for anti-inflammatory biologic therapy. A number of so-called TNF blockers have been produced, including infliximab (murine-human anti-TNF-α monoclonal antibody, Remicade, Centocor, Inc.), adalimumab (humanized anti-TNF-α monoclonal antibody, Humira, Abbott Laboratories), etanercept (p75-lgG1 fusion protein, Enbrel, Immunex, Corp.), and p55-lgG1 fusion protein (Therapeutic Antibody Centre, Oxford, UK). The TNF blockers have proven efficacy in the treatment of selected systemic inflammatory diseases, such as rheumatoid arthritis and Crohn's disease. While no randomized controlled trials exist to guide the role of TNF blockade in eye disease, numerous case reports and case series suggest certain of these drugs may be highly effective for recalcitrant ocular inflammation. Various forms of inflammatory eye disease may be particularly responsive, e.g. Behcet's uveitis. High cost and potentially life-threatening complications, including serious infections, are disadvantages of TNF blockade. At least one study highlights an unexpectedly high rate of complications in patients with uveitis treated with inflximab.

■ 2353

## Alpha Interferon therapy in Behcet's disease

*DEUTER C*
*Ophthalmology, Tuebingen*

**Purpose** In the last years the use of Interferon alpha (IFN-Purpose: In the last years the use of Interferon alpha (IFN-α) has changed the prognosis of ocular Behct´s Disease (BD).

**Methods** This presentation will analyse in a retrospective way the various IFN-α studies for ocular BD regarding response, visual acuity, treatment strategy with dosage, side effects and recurrence rate.

**Results** All studies with more then 10 patients report a complete remission rate of 80-92%. The visual acuity for the largest study group has recently been analysed on a 5-year basis, disclosing no visual loss in any patient. The rate of recurrences seems to depend on the therapy regimen and the dosage. Unfortunately there a various differences in this regimen. While some investigators had been added corticosteroids at the beginning in high dosages, others reduced it successfully to a lower dosage.

**Conclusion** IFN-α has been shown to be extraordinary effective in various uncontrolled studies. At the moment a controlled study against Cyclosporin A is under investigation.

■ 2354

## Overview of alternative pathways for biologic therapy

*DE SMET M*
*Ophthalmology, University of Amsterdam, Amsterdam*

**Purpose** Several novel agents have been developed which target specific signalling steps in the inflammatory cascade which can serve as targets for immune modulation. This presentation will explore currently promising options at various stages of investigation.

**Methods** Results from open label trials with anti-TNF agents were reviewed for efficacy and side effects. Medline search for novel biologic agents (antibodies) as well as immunomodulators was carried out. Novel drug delivery modalities were identified from the ARVO abstracts of 2005.

**Results** Anti-TNF agents are effective modulators of ocular inflammation, particularly when used for several months. Their ability to induce prolonged remission is variable but longer than that observed with most currently used medications. In view of their systemic toxicity, alternatives are required. Novel drug delivery systems are at various levels of development, and may obviate systemic toxicity. In addition some new biologic agents appear to have interesting properties such as the ability to limit neovascularization while inhibiting inflammation.

**Conclusion** The ability of inducing remission, limiting systemic or ocular toxicity, or the ability to provide additional biologic characteristics should be considered as important targets in assessing new biologic agents for clinical development.

■ 2355

## Gene therapy for ocular inflammatory disease

*ALI R*
*London*

ABSTRACT NOT PROVIDED

■ 2361

## Induction of RPE Differentiation Markers in Human Mesenchymal Stem Cells from Adipose Tissue

*VOSSMERBAEUMER U (1), KUEHL S (1, 2, 3), KERN S (2, 3), KLüTER H (2, 3), EICHLER H (2, 3), JONAS JB (4), BIEBACK K (2, 3)*
*(1) Dept. of Ophthalmology / University of Heidelberg, Faculty of Clinical Medicine, Mannheim*
*(2) Institute for Transfusion Medicine and Immunology, University of Heidelberg, Faculty of Clinical Medicine, Mannheim*
*(3) German Red Cross Blood Donor Service Baden-Württemberg - Hessen, Mannheim*
*(4) Dept. of Ophthalmology / University Eye Hospital, University of Heidelberg, Faculty of Clinical Medicine, Mannheim*

**Purpose** Mesenchymal stem cells (MSC) from adipose tissue have been shown to be differentiable into various cell types of the mesodermal lineage. We investigate the potential of such uncommited cells from adult human adipose tissue to be induced to differentiate into a phenotype displaying retinal pigment epithelium (RPE) characteristics.

**Methods** MSC from human lipoaspirate were cultured in MSC growth promoting medium. MSC properties were ascertained by assays for mesodermal differentiation and FACS analysis for stem cell specific surface antigen patterns. Differentiation towards RPE lineage was triggered by exposure to conditioned media from porcine RPE cells, and/or Vasoactive Intestinal Peptide (VIP). Resulting cell populations were assessed for expression of RPE-specific markers.

**Results** AT derived MSC were tested positive for an MSC typical array of CD markers. Their potential to differentiate along the mesodermal lineage was assessed using adipo-, osteo- and chondrogenic differentiation assays.Following culture of undifferentiated MSC with preconditioned medium and/or VIP, cells stained positive for RPE markers Bestrophin, Cytokeratine 8/18 and RPE 65. Results were equivalent for either of the three culture conditions.

**Conclusion** MSC from AT are shown to express RPE markers upon induction with either RPE conditioned medium and/or VIP. This alludes to transdifferentiation potential of MSC into neuroectodermal lineage, yielding cells with molecular characteristics of RPE cells. To date, this is the first report demonstrating this capacity. Further refinement of analysis could eventually point to novel options for a tissue regeneration therapy in degenerative macular disease.

■ 2362

## Induction of alphaB-Crystallin in optic nerve head astrocytes by TGF-beta and hypoxia

*YU AL (1), BURGER J (1), LäTJEN-DRECOLL E (2), KAMPIK A (1), WELGE-LÜßEN UC (1)*
*(1) Ophthalmology, Munich*
*(2) Anatomy, Erlangen*

**Purpose** Previous histological studies of glaucomatous optic nerves revealed an increased expression of the small heat shock protein alphaB-Crystallin in optic nerve astrocytes. In other cellular systems, this chaperone is inducible by transforming growth factor-beta (TGF-beta) and hypoxia. Both factors are discussed to be involved in the pathogenesis of primary open angle glaucoma. Therefore, the influence of these two factors on the expression of alphaB-Crystallin in cultured human optic nerve head (ONH) astrocytes was investigated.

**Methods** Astrocytes were isolated from eyes of 5 human donors and cultured monolayers were treated with 1.0 ng/ml TGF-beta2 for 12, 24 and 48 h or kept under hypoxic conditions from 4-24 hours with 24-48 h of reoxygenation. Expression of alphaB-Crystallin was examined by immunohistochemistry, northern and western blots.

**Results** TGF-beta2 treatment upregulated the expression of alphaB-Crystallin in cultured astrocytes. Fold inductions ranged between factor 2.5 to 4 on the mRNA level and 1.5 to 2 on the protein level. Hypoxia increased the expression of alphaB-Crystallin after 12 h of hypoxia about 1.8 fold on the mRNA and 1.4 on the protein level. Reoxygenation further increased the expression of alphaB-Crystallin about 3 fold on the mRNA and protein level. The other investigated time periods showed similar results.

**Conclusion** TGF-beta2 and hypoxia are capable of inducing the expression of the chaperone alphaB-Crystallin in cultured ONH astrocytes. It is tempting to speculate that both factors might be involved in the initiation of the modifications of astrocytes in glaucomatous ONH. Therefore blocking TGF-beta action and preventing hypoxia could help to minimize glaucomatous ONH changes.

■ 2363

## Human retinal microglia direct retinal progenitor cell proliferation and differentiation

*BALASUBRAMANIAM B, DICK AD, CARTER DA, MAYER E*
*Ophthalmology, Bristol*

**Purpose** During neurodegeneration or following inflammation there appears an inability to replace lost cells, despite evidence of retinal progenitor cells. Activated microglia (MG) cultured from adult human retinal tissue have been shown to respond to stimuli such as lipopolysaccharide and interferon gamma (LPS-INFg) by secreting cytokines. This impact of MG on regeneration or replacement has been shown in rodents where IL-6 suppresses neurogenesis. Therefore, we utilized the adult human retinal explant model, to further investigate the potential role of activated MG in directing human retinal progenitor cell proliferation.

**Methods** Human neurosensory retinal explants were cultured in DMEM alone and DMEM supplemented with either TNF-a, TGF-b, LPS-INFg or N2FGF. At day 4, the 0.5mm explants were removed and fresh medium was added to the growing MG cells. At Day 7, the medium of each culture was removed and concentrated for feeding retinal cell suspensions. Neurospheres were counted at day 7 and 14 before re-feeding. The MG generated cytokines were analysed by bead assays for IL-8, IL-1b, IL-6, IL-10, TNF and IL-12p70.

**Results** All MG conditioned media samples contained secreted IL-6 and IL-8 by day 7. Neurospheres of variable sizes were formed. Relative to DMEM alone and TNFa, cultures incubated with 1% N2FGF produced large neurospheres. By contrast, TGFb and LPS-INFg inhibited neurosphere growth.

**Conclusion** Activated adult human retinal microglial cells produced cytokines including IL-6 and IL-8 that suppressed neurosphere formation. This was reinforced by TGFb and LPS-INFg but partly reversed by N2FGF. Human retinal microglia may play a direct role in the proliferation and differentiation of retinal progenitor cells.

■ 2364

## Comparison of the in vitro expression profile of primary retinal ganglion cells and retinal cell lines, RGC-5 and R28

*DAVIES VJ (1), VOTRUBA M (1, 2)*
*(1) School of Optometry and Vision Sciences, Cardiff University, Cardiff*
*(2) Cardiff Eye Unit, University Hospital Wales, Cardiff*

**Purpose** Autosomal Dominant Optic Atrophy (ADOA), due to mutations in the OPA1 gene, is characterised primarily by cell death confined to the retinal ganglion cell (RGC) layer. The purpose of this study was to examine how closely transformed RGC lines reflect the characteristics of primary RGCs in terms of the retinal cell markers expressed, and thus their value to the study of ADOA pathophysiology.

**Methods** Primary RGCs were generated using immuno-magnetic selection of Thy-1 positive cells from P7 dissociated Wistar rat retinas, and grown on laminin coated 96-well plates. Cells from two retinal cell lines, RGC-5 and R28 were seeded on 4-well plates and grown in supplemented DMEM. Cells were fixed in 3.7% paraformaldyde and immunostained. The following retinal cell markers were examined. Thy 1 is a glycoprotein expressed highly in the RGC layer. Tuj 1 recognises βIII-tubulin, a cytoskeletal protein expressed early in development of RGCs. Brn-3b, a POU domain transcription factor is essential for the normal development of RGCs. Neurofilament subunits, NF-L, M and H, are neuronal cytoskeletal markers used to visualise RGCs. Tau and MAP-2 are cytoskeletal proteins, that mark axons and dendrites, respectively, and were used to assess cell polarity. Finally, OPA1 is expressed in RGCs and other cells of the inner retina.

**Results** Positive staining of the cell body, membrane and axons was observed in RGC-5 and primary RGCs for Thy 1, Tuj 1 and OPA1. This is compatible with staining patterns seen with immunohistochemistry of rat, mouse and human retina sections. The other markers require further clarification.

**Conclusion** The RGC-5 cell line is a valuable line for examining the cytotoxic mechanisms behind RGC loss in ADOA.

■ **2365 / 261**

## Expression of endostatin in human choroidal neovascular membranes secondary to age related macular degeneration

*TATAR O (1), ADAM A (2), ROHRBACH J Martin (1), LUCKE K (3), SHINODA K (4), BARTZ-SCHMIDT KU (1), GRISANTI S (1)*
*(1) Eberhard-Karls University, University Eye Clinic Department 1, Tuebingen*
*(2) Eberhard-Karls University, Department of Pathology, Tuebingen*
*(3) Eye Clinic Universitätsallee, Bremen*
*(4) Keio University, Department of Ophthalmology, Tokyo*

**Purpose** To investigate the expression of endostatin, an endogenous angiogenesis inhibitor, in human choroidal neovascular membranes (CNV) secondary to age related macular degeneration (AMD) with regard to vascularization and proliferative activity.

**Methods** Retrospective review of 36 patients who underwent removal of CNV. Thirty-six CNV were analyzed by light microscopic immunohistochemistry for CD34 (endothelial cells, EC), CD105 (activated EC), Ki-67 (cell proliferation), Cytokeratin 18 (epithelial cells), VEGF (vascular endothelial growth factor), E-selectin and endostatin. Donor eyes (n=7) including one with AMD were used as controls.

**Results** Endostatin immunostaining was present in choroidal vessels of five and in RPE cells and Bruch's membrane of two donor eyes without AMD. In the eye with AMD, endostatin was present in RPE, Bruch's membrane and choroidal vessels. Ninety-two percents (33/36) of CNV disclosed endostatin staining. RPE cells, choroidal vessels and stroma were positive in 50% (16/36), 72% (26/36), and 78% (28/36) of the membranes respectively. Both control eyes and CNV expressed all the investigated markers except E-selectin being positive only in membranes.

**Conclusion** Endostatin, an endogeneous angiogenesis inhibitor, is expressed in CNV. It is co-expressed with E-selectin that is required for its antiangiogenic activity only in CNV but not in control eyes. Therefore, endostatin may contribute to the involution process of the neovascularization in AMD.

■ **2366 / 262**

## Ultrastructural analysis of photoreceptor neurogenesis in the adult monkey and human far peripheral retina

*CUENCA N (1), MARTINEZ-NAVARRETE G (1), MARTIN-NIETO J (2), ANGULO A (3)*
*(1) Biotecnología, Alicante*
*(2) Fisiología, Genética y Microbiología, Alicante*
*(3) Interuniversitario de Óptica, Alicante*

**Purpose** In vitro, retinal stem cells and differentiating neurons have been identified in neurosphere cultures from the ciliary body epithelium of adult rodents and humans. We have examined at the ultrastructural level cones and rods residing in the far peripheral retina of adult primates, in order to determine whether they represent either differentiating or degenerating photoreceptors.

**Methods** Transmission electron microscopy was carried out on ultrathin sections of monkey peripheral retina. As well, cryostat vertical sections obtained from adult macaques and humans were subjected to immunostaining with specific antibodies against nestin and a set of phenotypic markers for rods and cones.

**Results** Cones and rods located at the peripheral retinal margin exhibit an immature ultrastructure, with tiny outer segments and a huge axon terminal. De novo formation of outer segment disk membranes at the upper portion of incipient ellipsoids was apparent from the fusion of Golgi vesicles. in the non-laminated retina. Along this zone, the sequential morphological development of cells expressing photoreceptor markers was accompanied by the establishment of synaptic contacts with horizontal and bipolar cells, in parallel to the growth in length of outer segments. However only two horizontal-cell processes could be identified at ribbon triad synapses.

**Conclusion** The far peripheral retina thus constitutes a region where neurogenesis of cone and rods takes place in a spatial-temporal fashion in adult primates. This process can be envisioned to have a role in the normal turnover of retinal circuitries that become lost along life in mammals.Support: MCyT BFI2003-01404, GV04B/452, ONCE and Fundaluce.

■ **2367 / 263**

## Alpha-Synuclein-containing synaptic ribbons, outer segment disks and desmosome-like junctions in the primate retina

*MARTINEZ-NAVARRETE G (1), ANGULO A (2), HERRERO MT (3), MARTIN-NIETO J (4), CUENCA N (1)*
*(1) Biotecnología, Alicante*
*(2) Interuniversitario de Óptica, Alicante*
*(3) Anatomía Humana y Psicobiología, Murcia*
*(4) Fisiología, Genética y Microbiología, Alicante*

**Purpose** Alpha-Synuclein is a presynaptic protein postulated to have a role in synapse maintenance and neural plasticity and neurodegenerative disorders. We have analyzed alpha-synuclein distribution in the distinct neuronal types of the retina, in order to obtain clues on its physiological role in the retina.

**Methods** We have used ABC preembedding immunocytochemical methods for electron microscopy and cryostat vertical sections of retinas from adult monkeys and humans for fluorescence microscopy. Immunostaining was carried out using with antibodies against alpha-synuclein and other markers specific for the distinct retinal neuronal types.

**Results** A strong immunoreactivity was found in the outer and inner plexiform layers. This protein immunolocalized to the cell bodies and dendrites from bipolar and amacrine cells. Ultrastructurally, alpha-synuclein located at desmosomes established between photoreceptors and Müller cells lining the outer limiting membrane. Immunostaining was also present in the outer segment disks from photoreceptors. At the level of pedicles and spherules alpha-synuclein was identified at synaptic ribbons and desmosome-like junctions between horizontal cells in the OPL.

**Conclusion** Alpha-Synuclein localization in photoreceptors suggests an involvement of this protein in outer segment disk formation and synaptic transmission at the OPL level. Roles in desmosome-like junctions, in the formation of the outer limiting membrane and/or the turnover of presynaptic vesicles can also be postulated. It is thus possible that mutations in the alpha-synuclein gene could be causative of retinosis pigmentosa. Support: MCyT BFI2003-01404, GV04B/452, ONCE and Fundaluce

## ▪ 2412
## Early detection and prevention of wet AMD

*SILVA R, FARIA DE ABREU JR, CUNHA VAZ JG*
*AIBILI, Coimbra*

**Purpose** The presence of large drusen and pigmentary changes increase the risk of developing neovascular lesions in patients with age-related macular degeneration (10 to 20% in 5 years). When the contralateral eye has an exudative lesion, the five-year risk for developing exudative AMD in the second eye increases to about 60%. Early detection of CNV has a capital importance for a more successful treatment. However, for preventive intervention, it is mandatory to identify which eyes will progress to wet AMD before first symptoms or lesions appear. The Amser Grid is a subjective tool with great value in the detection of metamorphopsia, one of the first signs of exudation. Fluorescein angiography and OCT are currently being used in the clinical practice to identify and classify AMD features. Other examens, like ICG and fundus autofluorescence may potentially contribute to the identification of high-risk eyes. The Scanning Laser Ophthalmoscope – Retinal Leakage Analyzer (SLO-RLA) joins functional and topography evaluations: outer blood-retinal barrier alterations can be evaluated in the high-risk contralateral eyes. High doses of vitamins have shown some efficacy in high-risk eyes and angiostatic drugs may also have a particular role preventing the evolution to wet AMD.

## ▪ 2413
## Pathophysiology of AMD - evolving concepts, new therapeutic targets

*DE SMET MD*
*Ophthalmology, University of Amsterdam, Amsterdam*

**Purpose** To review the pathophysiologic mechanisms involved in AMD and outline strategies for its modulation and control.

**Methods** A review of experimental and clinical data pertaining to AMD with an emphasis on mechanisms both early and late, the modulation of which can lead to therapeutic benefit.

**Results** A number of pathways appear relevant to AMD manifestations irrespective of the primary etiology. These include the sequelae of oxidative damage, RPE cell dysfunction, alterations in extracellular matrix and the associated deviant immunologic response, cell death, a disregulation in pro-angiogenic factors above its basal secretory level.

**Conclusion** Understanding the contributions made by various pathways to the development or modulation of AMD can lead to more focussed treatments. Combination or sequential therapies will likely maximize therapeutic benefit while minimizing side effects. Choice and timing of pharmacologic intervention will depend on a good understanding of pathophysiology.

## ▪ 2414
## Overview of clinical results with new therapies

*KOROBELNIK JF*
*University Hospital, Bordeaux*

**Purpose** This presentation will address the key clinical results in support of existing and new therapeutic agents for the treatment of exudative AMD. Visudyne® (verteporfin PDT) therapy represents the first generation of photodynamic therapy agents aimed at selective ablation of neovascular membranes. As next generation PDT agents are being clinically evaluated, therapeutic agents designed to pharmacologically interfere with biological processes leading to the development of choroidal neovascularization are being investigated. Various anti-VEGF therapies in different stages of clinical development will be presented along with key clinical data. Angiostatic agents representing other therapeutic approaches, such as anecortave acetate, an angiostatic steroid devoid of clinically relevant glucocorticoid activity will be reviewed from a clinical perspective. Finally, a host of next generation agents in early clinical development will be summarized, along with the advent of combination therapy.

## ▪ 2415
## Drug delivery and novel administration procedures

*LEYS A, ANECORTAVE ACETATE STUDY GROUPS*
*Dept. Ophthalmology, University Hospitals Leuven, Leuven*

**Purpose** The presentation will address the various drug delivery methods for anti-angiogenic agents that have been developed for the treatment of ocular neovascular disorders. The method of choice for delivering such agents will depend on the ocular tissue being targeted, the physio-chemical properties of the drug including its pharmacokinetic profile by the specified delivery method, and its mechanism of action with consideration to maintaining adequate safety, yet providing a therapeutic dose for an extended treatment duration. New anti-antiogenic therapies have been developed for treating disorders such as exudative age-related macular degeneration, whereby aberrant vessel growth in the choroid and retina has blinding consequences. Photodynamic therapy utilizing a photo-reactive dye such as verteporfin, or other angiostatic agents including squalamine and bevacizumab are administered to the patient by systemic administration. Intravitreal injections have been utilized for a wide variety of angiostatic agents typical of various anti-VEGF strategies. Extraocular delivery, such as posterior juxtascleral depot administration of anecortave acetate has been developed for long-term, targeted delivery to the macula. Intravitreal or sub-Tenon's injections of triamcinolone acetonide are usually administered in combination with other therapies. Recently the DEX PS DDS applicator system has been developed for slow release of dexamethasone to the posterior segment. Each of these techniques offers advantages making it suitable for the specific condition being treated.

■ 2421

## Cytokine-mediated Regulation of Extracellular Superoxide Dismutasein Normal and Diseased Human Keratocytes

*BEHNDIG AB (1), ÅSTRöM E (1), MARKLUND SL (2)*
*(1) Department of Clinical Science/Ophthalmology, Umeå University, Umeå*
*(2) Medical Biosciences/Clinical Chemistry, Umeå*

**Purpose** To study the mechanisms regulating corneal stromal extracellular superoxide dismutase (SOD3) synthesis in health and disease by quantifying the basal and cytokine-modified SOD3 synthesis in cultured human keratocytes from normal, bullous keratopathy and keratoconus (KC) corneas.

**Methods** Keratocytes were obtained from patients with keratoconus and bullous keratopathy, undergoing corneal transplantation, and from healthy donor corneas. The cell lines obtained were cultured in 10% fetal calf serum until confluency. With the serum concentration reduced to 0.5%, a range of cytokines was added . SOD3 protein was determined in the media with an ELISA after 24, 48, 72 and 96h. To check for cytotoxicity, the leakage of lactate dehydrogenase at 96h was quantified. Also, the amounts of nitric oxide end products (NOX) in the media were determined.

**Results** Platelet-derived growth factor (PDGF) induced a general reduction in SOD3 synthesis, compared to untreated controls ($P<0.05$). Interleukin-1α (IL-1α) had an inhibitory effect on SOD3 synthesis exclusively in the keratoconus keratocytes ($P<0.01$). NOX levels were also lowered in KC keratocytes after the addition of IL-1α. No signs of unspecific cytotoxicity were seen.

**Conclusion** We here demonstrate that SOD3 synthesis in human keratocytes is down regulated by PDGF, and that KC keratocytes respond with a reduced SOD3 synthesis to IL-1α, which is not the case in normal or bullous keratopathy karatocytes. Since IL-1α is up regulated in corneal trauma or inflammation, KC corneas may muster an insufficient oxidative defense under such conditions. Possible mechanisms behind the reduced NOX levels with IL-1α in KC are discussed.

■ 2422

## Corneal thinning in keratoconus

*MEEK KM (1), BOOTE C (1), HAYES S (1), TUFT S (2), BRON AJ (3)*
*(1) Optometry and Vision Sciences, Cardiff*
*(2) Moorfields Eye Hospital, London*
*(3) Nuffield epartment of Ophthalmology, Oxford*

**Purpose** To map the lamellar organisation across the normal and keratoconic human cornea and to provide an explanation for corneal thinning and ectasia.

**Methods** Synchrotron x-ray diffraction was used to quantify the projected two-dimensional distribution of lamellae (in the form of the orientation and mass of total and preferentialy aligned collagen) at regular intervals across human and keratoconus corneas.

**Results** Human corneas show a symmetrical distribution of total collagen increasing from the central to the peripheral cornea. However, there is a non-rotationally symmetrical distribution of preferentially aligned lamellae, which consist of at least two separate populations, a central inferior/superior and medial/lateral population and a set of anchoring lamella that enter and leave the peripheral cornea avoiding the prepupillary zone. Furthermore, left and right eyes are not identical but show symmetry about the central body line. In keratoconus, both sets of preferentially aligned lamellae are distorted. The distortions can be related to the topography of the corneas.

**Conclusion** Keratoconus involves a high degree of slippage between collagen fibrils and/or between lamellae. Lamellar bifurcations imply that collagen fibrils may not follow a direct route from limbus to limbus. Bifurcation points are potentially weak spots from which tears can propagate should the interfibrillar coherence be compromised, allowing collagen reorientation, slippage and tissue thinning.

■ 2423

## Protection of endothelial cell death against oxidative stress during acute corneal graft rejection

*BOURGES J-L (1, 2, 3), VALAMANESH F (2), RENARD G (4), DEKOZAK Y (2), BENEZRA D (5, 2), BEHAR-COHEN F (2, 6)*
*(1) Hotel-Dieu Hospital, Paris*
*(2) U598, INSERM, Paris*
*(3) Paris-Descartes University, Paris*
*(4) Hôtel-Dieu Hospital, Paris*
*(5) Ophthalmology, Hadassah Hospital, Jerusalem*
*(6) Ophthalmology, Rothschild Ophthalmic Foundation, Paris*

**Purpose** To test the protective effect of an inhibitor of inducible nitric oxide synthase (1400W), against corneal grafted endothelial cells (EC) death during acute rejection.

**Methods** In model of acute rejection after penetrating keratoplasty (PK), two groups of rats (n=11) were randomized, receiving either systemic injections of saline (group I, n=5) or 0.5 mg/kg/day 1400W (group II; n=6). The rejection process was assessed using three clinical scores: corneal edema, opacity or neovascularisation. Rats were sacrificed on day 13 after PK. The recipient bed and the transplanted graft were Flat–mounted and stained with DAPI and phalloidin. The aqueous humor was pooled in each group to dose the nitrite level.

**Results** A sparing of the EC in group II was observed, but no significant difference was observed in clinical scores of rejection. The mean EC count/field (ECC) was 237cells (+/–84.7) on the grafts and 433 cells (+/–32.65) on the recipient corneas in group I ($p=0.008$). In group II, ECC was respectively 397 cells (+/–107) and 551 cells (+/–42.2)(NS, $p>0.1$). The ECC on the recipient corneas was not different in both groups ($p=0.33$), wereas the ECC in the graft corneas was significantly higher in the group II ($p<0.05$). In group I corneas, larger areas did not stain with DAPI/Phalloidin illustrating the total loss of EC. Nitrite levels were higher in the aqueous humor of saline injected rats than in treated rats.

**Conclusion** Treatment with iNOS inhibitor can reduce markedly the EC loss in the graft during the rejection process and may prevent it during corneal graft rejection.

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## Modified Microkeratome-Assisted Posterior Lamellar Keratoplasty Using A Tissue Adhesive

*BEHRENS A (1), PIROUZMANESH A (1), HERRETES S (1), REYES JMG (1), SUWAN-APICHON O (1), CHUCK RS (1), WANG DA (2, 3), ELISSEEFF JH (2, 3), STARK WJ (1)*
*(1) The Wilmer Ophthalmological Institute, Johns Hopkins University, Baltimore*
*(2) Departments of Cell Biology, Johns Hopkins University, Baltimore*
*(3) Biomedical Engineering, Johns Hopkins University, Baltimore*

**Purpose** To compare graft stability and astigmatic change using suture vs. tissue adhesive in an experimental model of microkeratome-assisted posterior lamellar keratoplasty (PLK).

**Methods** A 300-µm thick flap-keratectomy after incomplete pass was performed in human donor corneoscleral rims using an artificial anterior chamber and a manual microkeratome. The flap hinge at the left central opening border, providing a wide hinge to add stability. After flap reflection, a 6.25 mm trephination was performed obtaining a disc of posterior stroma, Descemet's membrane, and endothelium. The disc was positioned in a sutureless fashion, and the flap secured with either 5 interrupted sutures, or a chondroitin-sulfate-aldehyde based adhesive. Increasing intrachamber pressures were created detecting graft stability. Videokeratographic data was recorded evaluating astigmatic change.

**Results** The mean astigmatic change was 3.08 D (0.84) in the sutured group, and 1.13 D (0.55) in the glued group ($p=0.008$). Mean resisted pressures were 95.68 (27.38) mmHg and 82.45 (18.40) mmHg in the sutured and glued groups, respectively ($p=0.97$).

**Conclusion** This modified technique of microkeratome-assisted PLK showed excellent graft stability in both groups. Flaps sealed with the novel tissue adhesive had reduced astigmatic changes in our experimental model.

## ▪ 2425 / 226

### Keratocyte apoptosis in keratoconus: A function of TIMP-3 and TIMP-1 synthesis?

*SMITH VA (1), MATTHEWS FJ (2)*
*(1) Ophthalmology Unit, Bristol*
*(2) Department of Virology, Bristol*

**Purpose** The thinning of keratoconic corneas could result from keratocyte apoptosis or be induced or perpetuated by the activation of matrix degrading enzymes, particularly those of the MMP family. The MMP inhibitors TIMP-3 and TIMP-1 may exhibit apoptotic and anti-apoptotic properties respectively. Because of their potential to influence keratoconus progression, the effects of these proteins on keratocyte viability were investigated.

**Methods** Keratocyte cultures were infected with RAdTIMP-3 and RAdTIMP-1. The expressed proteins were quantified by ELISA. Apoptotic cells were detected by TUNEL and caspase-3 activity. The anti-apoptotic effects of TIMP-1 were investigated by RAdTIMP-1 and RAdTIMP-3 coinfection and by adding TIMP-1 protein to keratocyte cultures prior to infection with RAdTIMP-3. Immunohistochemistry was used to localise and determine relative numbers of apoptotic and TIMP producing keratocytes in normal and keratoconic corneal sections.

**Results** TIMP-3 over-expression induced keratocyte apoptosis. Upregulated TIMP-1 production or the addition of exogenous TIMP-1 protein prevented keratocyte overgrowth, changed keratocyte morphology and reduced the extent of TIMP-3 induced apoptosis. In vivo significantly more apoptotic cells were identified in the anterior stroma of keratoconic corneas than normal corneas and the majority of theTIMP-3 and TIMP-1 producing keratocytes were also located in this region.

**Conclusion** TIMP-3 over-expression induced apoptosis in keratocytes cultured from normal corneas. TIMP-1 protected these cells against TIMP-3 induced apoptosis. Localised relative concentrations of TIMP-3 / TIMP-1 may thus determine whether a keratocyte becomes apoptotic or remains viable. This may be relevant to the keratoconic condition.

## ▪ 2426 / 227

### Agreement between two non-contact specular microscopes: TOPCON SP2000 versus RHINETEC

*THURET G (1, 2), NGUYEN F (1), ZHAO M (2), DEB N (2), GAVET Y (3, 2), GAIN P (1, 2)*
*(1) Ophthalmology dept, University hospital, Saint-Etienne*
*(2) Lab 'Cell survival and adhesion in cancer and graft' EA3063, faculty of medicine, Saint-Etienne*
*(3) Ecole Nationale Superieure des Mines, Saint-Etienne*

**Purpose** The new Rhinetec non-contact specular microscope (Germany) determines endothelial cell density (ECD) by a cell center method, either automatically or after manual touch-up. Aim: to determine its agreement with the widely widespread Topcon SP-2000 (Tokyo, Japan). This later is based on cell boundaries detection and requires extensive manual corrections of cell contours to be valid

**Methods** Successive assessments of the central ECD of 229 eyes were performed by a single ophthalmologist (FN). The Bland Altman method was used to determine the agreement between Topcon with manual corrections and Rhinetec with the automatic method and with manual corrections

**Results** Among the 229 corneas, 174 where form healthy eyes, 41 post graft, 7 post cataract surgery and 7 other. Mean age was 49 (11 to 93), median 45. ECD (Topcon touched-up) ranged from 476 to 3263 cells/mm$^2$. Numbers of counted cells were similar: 137+/-40 (Topcon) vs 142+/-42 (Rhinetec) p=0.067. Agreement between automatic Rhinetec and touched-up Topcon was poor, with a mean difference of 190 cells/mm$^2$ (Rhinetec>Topcon) with limits between -636 and +912 cells/mm$^2$. This automatic method overestimated low ECD (<1500) and underestimated high ECD (>2500). Agreement between both manual methods was high with a mean difference of 12 cells/mm$^2$ (Rhinetec>Topcon) and limits set between -402 and +427 cells/mm$^2$. The tendency to overestimated low cell density and underestimate high slightly persisted

**Conclusion** Full automatic methods must definitely be rejected. Rhinetec shows a good agreement with Topcon SP2000 when extensive manual corrections are performed. Nevertheless, regarding the two bounds of agreements on this sample, one must accept a possible consistent individual variation

## ▪ 2427 / 228

### Osteo-odonto Keratoprosthesis: A 40-Year Review

*MICHAEL R (1), SRIPATTANAWAT R (1), ALVAREZ A (1), TEMPRANO J (2), DE LA PAZ MF (2), CHAROENROOK V (2), ALVAREZ DE TOLEDO J (2), BARRAQUER RI (2, 1)*
*(1) Institut Universitari Barraquer, Barcelona*
*(2) Centro de Oftalmologia Barraquer, Barcelona*

**Purpose** To study short and long term functional results after Osteo-Odonto Keratoprosthesis (OOKP).

**Methods** We reviewed the charts of 287 patients who underwent osteo-odonto keratoprosthesis at the Barraquer Centre of Ophthalmology from January 1964 to May 2005. A total of 337 cases, 100 of which were bilateral were reviewed; 219 were male and 116 were female. All surgeries were done by a single surgeon using Strampelli´s technique with a variation using tibia (Temprano's technique) if patient was edentulous. Functional success was defined as visual acuity > 0.05 (legal blindness as defined by the WHO). Survival rates were calculated using life tables and the Kaplan-Meier estimator.

**Results** Mean follow-up time was 74 months (range: 1-585 months). Mean age of patients was 42 years (range: 4 – 86 years). Prior to intervention all patients were legally blind (VA < 0.05) and at final follow-up 153 patients had visual acuity better than 0.05. Overall survival rate was 86% at 1 month, 71% at 1 year, 50% in 5 years, 38% in 10 years and 18% in 25 years. Complications encountered were cataract (61%), vitritis (36%), expulsion (35%), glaucoma (19%), aseptic necrosis (19%), retinal detachment (16%). Survival rates for chemical burn (135 cases), Stevens-Johnson Syndrome (39 cases), Ocular cicatricial pemphigoid (16 cases) and other aetiologies are presented.

**Conclusion** A 50% functional success rate after 5 years is quite satisfactory for these end-stage ocular-surface diseases.

## ▪ 2428 / 229

### The assessment of corneal endothelium before and after cornea storage in tissue culture conditions

*JIRSOVA K, TOMSU S, ANDRLE J, FILIPEC M*
*Laboratory and Ocular Tissue Bank, Dept of Ophthalmology, General Teaching Hospital and Charles University, Prague*

**Purpose** The aim of this study was to compare the endothelial cell density, the mean number of live/dead cells per mm2, the coefficient of variation and the degree of hexagonality before and after preservation of human corneas under tissue culture (TC) conditions.

**Methods** Photographs of the central part of 120 corneas, taken before and after storage in tissue culture at 31°C for periods ranging from one to four weeks, were analysed. The endothelial cell density (ECD) and the number of dead cells per mm2 were calculated from phase contrast and bright field photographs, respectively. All photographs were encoded and the assessment was performed separately by two experienced observers using a Lucia computer analysis system. The data from all storage periods were combined and processed by the same system to determine the following parameters: endothelial cell density, dead endothelial cell density (D-ECD), live endothelial cell density (L-ECD), coefficient of variation of cell area, (CV) and percentage of hexagonal cells, (6A).

**Results** The mean ECD before cultivation was 2773 cells/mm2, L-ECD was 2728 cells/mm2 and D-ECD was 45 cells/mm2. During the cultivation, the mean ECD value decreased to 95%, L-ECD to 96% and D-ECD to 16% of original values. The degree of hexagonality decreased from 0.54 before, to 0.51 after storage. The decreases of all these parameters were significant, while polymeghatism was the only value that was not significantly changed (0.20 before and after TC).

**Conclusion** The significant decrease in the number of dead cells during TC storage up to 5 weeks was observed. This leads to the conclusion that the TC method is especially indicated for the preservation of corneas with higher numbers of dead cells.

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## The presence of HLA-DR positive cells in human corneas stored under hypothermic or tissue culture conditions

*AL FAKIH A (1), JIRSOVA K (1), FILIPEC M (2)*
*(1) Laboratory and Ocular Tissue Bank,Deparment of ophthalmology, Prague*
*(2) Ophthalmology, Prague*

**Purpose** To compare the number of HLA-DR positive cells in fresh corneo-scleral discs and in discs stored under hypothermic or tissue culture (TC) conditions.

**Methods** Corneo-scleral discs excluded from the transplant program were used. Four unpreserved corneo-scleral discs, 16 stored in hypothermic (Optisol-GS,4°C), and 20 stored in TC (31°C) for 3-5, 7-9, 12-14, and 20-24 days, and 28 days (for TC only) were used. All discs were dissected into four concentric zones: central, pericentral, peripheral cornea and limbo–scleral zone. The tissue was snap-frozen in liquid nitrogen and stored at -70°C until processed. 7 µm thick sections were fixed and streptavidin-biotin complex/alkaline phosphatase indirect imunohistochemistry using anti HLA-DR (Immunotech, France) was performed. HLA-DR positive cells were counted using a Lucia analysis system.

**Results** The number of HLA-DR positive cells detected in the epithelium was 3 cells/mm² in central and pericentral cornea, 7 cells/mm² in periphery, 20 cells/mm² in limbal, and 33 cells/mm² in conjunctival epithelium. A comparable decrease in the number of HLA-DR positive cells were detected in epithelium of corneo-scleral discs stored in hypothermic and TC conditions for 3-5 days. Complete absence of HLA-DR positive cells was observed after 12-14 days in hypothermic and 7-9 days in TC conditions. The disappearance of HLA-DR positive cells in limbus and conjunctiva occurred after 20-24 days and more than 28 in hypotermic or TC storage, respectively.

**Conclusion** The decrease of HLA-DR positive cells is more pronounced in TC compared to hypothermic storage. The absence of HLA-DR cells in corneas stored in TC longer than 7 days could be favorable in preventing corneal graft rejection.

## ▪ 2431
## Unusual presentations of giant cell arteritis

*BOSCHI A*
*Ophthalmology, Brussels*

**Purpose** Giant cell arteritis (GCA) is a vision threatening disease. Visual loss, usually unilateral and due to optic nerve ischemia, is generally preceded by systemic symptoms as fever, weight loss, head pain, scalp tenderness, and jaw claudication and go with blood inflammatory signs. Systemic and visual manifestations usually respond to corticotherapy. But, atypical neuro-ophthalmological presentations in GCA have been reported.

**Methods** Discussion of different clinical cases will be presented.

**Results** Transient visual loss frequently precedes permanent visual loss, but exceptionally may constitute the only visual symptoms and this for several months. Besides, asymptomatic choroidal and optic disk vascular insufficiency has been reported in several cases. Controlateral optic nerve involvement might develop in +/_ 50% of cases, usually a few days later if patient are left untreated. However, controlateral optic nerve involvement might happen while patient is on oral or IV corticosteroid. A potential explanation for the progression of visual or neurological symptoms despite treatment is a propagating thrombus, probably favoured by platelets elevation induced by corticosteroids. The laboratory tests with the highest specificity for GCA are ESR and CRP. Nevertheless, absence of inflammatory syndrome has been described in +/- 3% of GCA cases. Corticosteroids are the gold standard treatment of GCA. Usually, corticosteroid allows stabilization of the visual deficit, only a very small percentage of some visual recovery has been reported.

**Conclusion** GCA is a puzzling disease. Practitioners have to be aware of its high variability in clinical manifestations, because prompt diagnosis is mandatory in avoiding irreversible visual deficit.

## ▪ 2432
## Mucormycosis:manifestations, diagnosis and treatment

*BORRUAT FX*
*Hopital Ophtalmique Jules Gonin, Neuro-Ophthalmology, Lausanne*

**Purpose** Mucormycosis is an infection by fungi (Absidia, Mucor, Rhizomucor or Rhizopus) which are broad, non-septate hyphae, branching at right angles. These organisms are ubiquitous, very rarely producing disease in healthy human subjects. Risk factors to develop an infection include mainly diabetes mellitus, especially with ketoacidosis, steroid or immunosuppressive therapy, renal disease, hemodialysis and deferoxamine therapy, and intravenous use of illicit substances. The hyphae have a special affinity for blood vessel walls and either invade them, proliferate and induce thrombosis, hemorrhage and ischemic necrosis, or will result in intracranial arterial aneurysm. Clinical presentation will depend on the primary site of infection and the ischemic complications induced by vascular thrombosis. The rhinocerebral form of mucormycosis is suspected in the presence of orbital cellulitis, orbital apex syndrome, cavernous sinus thrombosis or internal carotid artery occlusion in a patient with the aforementioned risk factors. The central nervous system form of mucormycosis is less frequent and presents with decreased level of consciousness, coma, cavernous sinus or internal carotid artery thrombosis, or cranial neuropathy.Diagnosis should be suspected in any debilitated, injured, immunocompromised, or diabetic patient presenting with one of these clinical manifestations. Confirmation of the diagnosis can be only made by tissue biopsy and culture.Untreated, mucormycosis results in death in more than 50% of cases. Early recognition and aggressive adequate therapeutic decisions are associated with a better general prognosis.This presentation will review the pathophysiology, the clinical presentations, and an updated therapeutic management of mucormycosis.

## ▪ 2433
## Toxic and Metabolic Neuro-Ophthalmic Emergencies

*MILEA D*

**Purpose** Several potentially life-thretening neuro-ophthalmological signs may be the presenting feature of systemic toxic or metabolic disorders emergencies, prompting their rapid diagnosis. These neuro-ophthalmological conditions may be isolated or associated with other general signs, their expression being most often heterogenous and non-specific, such as optic neuropathies, central vision dysfunction, pupillary disorders, cranial neuropathies, or abnormal eye movents. We will review the main toxic and metabolic causes of neuro-ophthalmic emergencies, such as botulism, optic neuropathies due to accidental or purposeful poisoning, systemic drug-induced ocular side effects, toxic or nutritional encephalopathies, as well as osmotic disorders.Type B botulism may occur after poisoning with Clostridium botulinum, the ophthalmological effects of its neurotoxin being typically the earliest manifestations of the disease, prior to life-threatening descending flaccid paralysis and autonomic nervous system dysfunction. Bilateral ophthalmoplegia or vertical nystagmus may be the presenting sign in thiamine deficiency causing Wernicke's encephalopathy, which can present in patients with otherwise normal mentation, prompting its early recognition. Consumption of small amounts of methanol or ethylene glycol can lead to severe vision loss or death. Despite rapid systemic symptomatic treatment, permanent, severe vision loss may eventually occur. At a higher level, cortical blindness may be a systemic drug-induced ocular side effect, related to ciclosporin or various other chemotherapies, such as vincristine.Appropriate management of these neuro-ophthalmic conditions requires their early recognition, since they may present in the course of daily practice.

## ▪ 2434
## Acute Third Nerve Palsy: who needs an arteriogram?

*KAWASAKI A*
*Neuro-ophthalmology, Lausanne*

**Purpose** The neuro-ophthalmologic emergency in a patient who presents with an acute, neurologically isolated third nerve palsy is the exclusion of an intracranial aneurysm as the etiologic cause of the third nerve palsy. Certain clinical features are associated with a greater likelihood of harbouring an underlying aneurysm. These include: age between 20 and 50 years, absence of vascular risk factors such as diabetes or hypertension, and the presence of a dilated and sluggishly reacting pupil on the side of the third nerve palsy. The gold standard test for diagnosing an aneurysm remains conventional arteriography, however this test carries a small risk of complication e.g. stroke, particularly in older persons. With the rapidly improving sensitivity of MR angiography or CT angiography for aneurysm detection, these non-invasive tests may be sufficient for ruling out aneurysm in some patients with third nerve palsy who are considered to have a relatively low risk for an underlying intracranial aneurysm . This presentation will review the clinical features and differential diagnosis of an acute third nerve palsy and provide recommendations for the appropriate use of non-invasive imaging versus invasive (conventional) angiography to rule out intracranial aneurysm.

## ▪ 2435
## Painful Horner's Syndrome

*KAWASAKI A*
*Neuro-ophthalmology, Lausanne*

**Purpose** Horner's syndrome is suspected when a patient has facial anhidrosis, ptosis and pupillary miosis. Horner's syndrome is due to injury of the sympathetic pathway to the head and eye, and a variety of lesions in a variety of locations may disrupt this long and circuitous pathway. A history of pain accompanying a Horner's syndrome is a red flag for an underlying lesion that may have serious morbidity or even mortality. The location and nature of the pain can be helpful in identifying the site of the underlying lesion. In a patient with Horner's syndrome, a history of pain above the neck is typically due to a lesion along the postganglionic portion of the oculosympathetic pathway (neck, skull base, cavernous sinus) whereas pain below the neck is likely a preganglionic lesion in the lungs or chest. Of the different causes of a painful Horner's syndrome, a carotid artery dissection is the most urgent diagnosis for exclusion because of the risk for stroke, particularly in the initial weeks after symptom onset. This presentation will review the evaluation of a patient with painful Horner's syndrome and focus on the diagnosis and management of acute carotid dissection.

## ▪ 2441
## Multiphoton microscopy

*KOENIG K (1), RIEMANN I (2), HUSS C (3), KRAUSE M (3), BAGOLII W (4), LEHARZIC R (2, 5)*
*(1) Friedrich Schiller University Jena & Fraunhofer IBMT, St. Ingbert*
*(2) Fraunhofer IBMT, St. Ingbert*
*(3) Universitäts-Augenklinik, Homburg*
*(4) Institute of Anatomy / Anatomy II, University of Jena, Jena*
*(5) JenLab GmbH, Jena*

**Purpose** Multiphoton imaging is based on two-proton excitation of NADH and flavins as well as on second har-monic generation of the extracellular matrix molecule collagen.

**Methods** We report on the use of 1 nanojoule near infrared 80 MHz femtosecond laser pulses for highly precise corneal refractive surgery and high-resolution imaging of endogenous fluorophores. Imaging was performed at GW/cm2 laser intensities whereas nanoprocessing was carried out at transcient TW/cm2 intensities.

**Results** Corneal imaging with a lateral resolution of 300 nm and an axial resolution of 1 µm was achieved when using objectives of high numerical aperture of 1.3. Histological examination reveals a minimum corneal cut size below 1 µm without destructive effects to sur-rounding tissues.

**Conclusion** femtosecond laser pulses of non-amplified laser systems provide novel tools for ocular nanosurgery as well as for diagnostics based on optical biopsies.

## ▪ 2442
## Wide field White Light OCT

*BOCCARA AC*
*Laboratoire d'Optique Physique, Ecole Supérieure de Physique et Chimie Industrielles, Centre National de la Recherche Scientifique, Paris*

**Purpose** Ultrahigh-resolution, full-field optical coherence tomography (OCT), which uses a white light source, allows bidimensional, noninvasive tomographic imaging without scanning. The goal of the present study was to apply full-field OCT to ocular tissue imaging in an attempt to explore the capabilities of the technique

**Methods** This full-field OCT system uses a Linnik-type interferometer with a tungsten-halogen source. The spatial resolution is 0.9 x 0.7 µm (transverse x axial). Unstained tissue samples (cornea, lens, retina, choroid, and sclera) and whole, unfixed eyes of rat, mouse, and pig were examined under immersion.

**Results** Cellular-level resolution was achieved in isolated tissue samples. En face (x-y) images revealed corneal epithelial and stromal cells, lens fibers, nerve fibers, major vessels, and retinal pigment epithelial cells. In x-z reconstructions, cellular layers within the cornea and retina and arterioles and venules were clearly defined.

**Conclusion** Ultrahigh-resolution, full-field OCT allows cellular-level imaging of unstained ocular tissues with high penetration depth. Although the current system is unsuitable for clinical use, this simple technique has potential for in vivo ocular examination, for which a new system is currently under development.

## ▪ 2443
## Multi-piece accommodating IOLs

*HO A*
*Vision Cooperative Research Centre, University of New South Wales, Sydney*

**Purpose** In this presentation, the theoretical accommodative results of single and multi-piece accommocative lenses, and future lens-refilling techniques will be exposed, and compared with current treatments for presbyopia

## ▪ 2444
## fs-laser induced elasticity changes to improve presbyopic lens accommodation

*LUBATSCHOWSKI H*
*Laser Zentrum Hannover, Hannover*

**Purpose** According to Helmholtz' theory of accommodation one of the mayor reasons for the development of presbyopia is the increasing sclerosis of the lens. One concept to delay the process of sclerosis or even regain the deformation ability of the lens might be the treatment of the lens by femtosecond laser pulses. Our aim was to evaluate appropriate laser parameters for this possible treatment and to analyse potential changes in deformation ability of the treated lenses.

**Methods** We performed different cutting patterns in enucleated porcine lenses (ex vivo) using the disruptive effect of an ultrafast near-infrared laser induced optical breakdown. Pulse energies and spot separationof the laser pulses were varied to investigate the effect on the generated cut. For an evaluation of the gain in deformation ability the lenses were rotated before and after treatment and the changes in the lens' diameter due to centrifugal forces were measured.

**Results** A smooth cutting was possible with appropriate parameters. The experiments showed an increase of elasticity in 70% of the eyes. When the lenses were treated subsequently, an average deformation ability increase of nearly 20%, determined by the change of thickness between untreated and treated lens, was measured.

**Conclusion** Femto-second laser lens treatment could be a possible way to treat or reduce presbyopic changes

■ **2445**

## Fourier domain OCT : high resolution 3D imaging

*WOJTKOWSKI M, KOWALCZYK A*
*Institute of Physics, Nicolaus Copernicus University, Torun*

**Purpose** Optical Coherence Tomography (OCT) technique is able to provide three-dimensional information about the retinal morphology similar to other tomographic methods including CT and MRI. However, acquisition speed of commercially available OCT instruments is insufficient to measure full sets of 3-dimensional data having a large number of pixels per image. In order to construct OCT instruments capable of collecting three dimensional sets of cross-sectional images with pixel counts similar to Stratus OCT, the acquisition speed should be increased by at least 50 times compared to the commercial unit. It has been demonstrated recently, that the novel application of Fourier domain detection to OCT technology significantly improves the speed and sensitivity of OCT instruments. These novel devices enable OCT imaging 60 times faster than commercial Stratus OCT. Such a speed is sufficient to measure three dimensional data in vivo. A combination of Fourier domain detection and commercially available, low cost, broad-band light sources enables improving three to five times the axial resolution of these novel OCT instruments comparing to Stratus OCT. In this talk three-dimensional, high resolution Fourier domain ophthalmic imaging will be presented and examples of qualitative data analysis will be shown.

■ **2446**

## Adaptic optics for imaging the retina

*FERNANDEZ EJ (1, 2)*
*(1) Center for Biomedical Engineering and Physics, Vienna University of Medicine, Vienna*
*(2) Laboratorio de Optica, University of Murcia, Murcia*

**Purpose** To increase transversal resolution and contrast of retinal images obtained in vivo by combining adaptive optics with two different ophthalmic techniques: scanning laser ophthalmoscopy and optical coherence tomography.

**Methods** The human eye is affected by optical aberrations, degrading the optical quality of retinal images. The resolution of the different ophthalmic techniques, as scanning laser ophthalmoscopy and optical coherence tomography, is limited by the effect of these aberrations. In this context, adaptive optics has recently been demonstrated for measurement and correction of ocular aberrations in real time. A wave-front sensor and a correcting device basically compound the adaptive optics system. The Hartmann-Shack wave-front sensor is the preferred one for ophthalmic applications. Nevertheless, different correcting devices have been proposed and demonstrated: deformable mirrors and liquid crystal spatial light modulators. The benefits and applications of these two different correcting technologies are studied. The combination of adaptive optics with scanning laser ophthalmoscopy and optical coherence tomography are presented.

**Results** Scanning laser ophthalmoscopes and ophthalmic optical coherence tomography combined with adaptive optics enable to obtain retinal images with unprecedented resolution and contrast in the living eye. Individual cells, as photoreceptors, and other interesting intraretinal features are resolved by using adaptive optics with these two imaging modalities.

**Conclusion** Although still under development, the combination of adaptive optics with the two different ophthalmic techniques presented in this work may enable early diagnosis and better understanding of several retinal pathologies, making in the future the use of this technology very interesting for clinics.

■ 2452
## Epidemiology of paediatric uveitis

*TUGAL-TUTKUN I*

**Purpose** To review the prevalence and patterns of uveitis in children.

**Methods** Published series of pediatric uveitis were reviewed.

**Results** Population-based surveys have shown that the incidence of uveitis in children is fivefold lower than in adults. Children account for less than 10% of uveitis cases seen at referral centers. The causes of uveitis in children also differ from the causes of uveitis in adults. While there are unique forms of uveitis and masquerade syndromes in childhood, some other entities often seen in adulthood are rare in children. There are also geographical differences in the incidence of various etiologies.

**Conclusion** Clinic-based studies suggest a high rate of complications and poor visual prognosis in a high percentage of children with uveitis.

■ 2453
## Diagnosis and investigation of paediatric uveitis

*DAVIES J*

**ABSTRACT NOT PROVIDED**

■ 2454
## Paediatric Behçet's uveitis

*TUGAL-TUTKUN I*

**Purpose** To describe the demographic and clinical features of childhood-onset Behçet uveitis.

**Methods** A retrospective analysis was made of 36 patients with onset of Behçet uveitis at 16 years of age or younger.

**Results** Twenty-five patients were male, 11 were female. Uveitis was the initial symptom in 8%. Mean age at onset of uveitis was 13.6 years. Bilateral panuveitis with retinal vasculitis and retinitis was the most common form of ocular involvement. Cataract, maculopathy, and optic atrophy were the most common complications. Immunosuppressive therapy was administered to 75% of the patients. Response to treatment was variable. Six patients were legally blind at the time of last visit.

**Conclusion** Similar to the adult group, there are individual variations in disease course and severity in pediatric patients with Behçet disease.

■ 2455
## Current knowledge on uveitis related to juvenile idiopathic arthritis

*PLEYER U (1), KEITZER R (2)*
*(1) Dept. of Ophthalmology, Charité, Berlin*
*(2) Dept. of Pediatric Pneumology and Immunology, University Medicine Berlin, Humboldt University, Berlin*

**Purpose** Uveitis is a serious extraarticular manifestation of juvenile idiopathic arthritis (JIA). Because of its indigenous onset, high complication rate and risks as a potentially blinding disorder at young age, uveitis in JIA remains a challenge for ophthalmologists. Interestingly some concepts based on clinical and laboratory finding have been changed and should receive more attention.Whereas gender (girls) and type of arthritis (oligoarthritis) have been previously suggested as risk factors for uveitis, recent prospective studies could not confirm this and indicate the need for refinements in screening efforts. Conditions that are often predictive of poor outcome due to uveitis include: gender (boys), early onset of arthritis and short time interval between onset of arthritis and uveitis and the severity of intraocular inflammation at the first presentation.The pathogenic mechanisms remain unclear, but an association with antinuclear antibodies is well-known. A variety of autoantigens including antinuclear antibodies, anti-histon 3, collagen- type II and heat shock protein 60 (hsp60) have been suggested to play a role in JIA-associated uveitis but still need to be confirmed in independent studies. The role of cytokines in JIA is an active field of interest. TNF-α, INF-γ and their receptors are significantly elevated in synovial tissue of children with JIA. New treatment options directed against these cytokines have been successfully introduced for arthritis, however their value for uveitis is not jet clear.Taken together, screening of children with JCA for uveitis and identifing confounding factors that correlate with intraocular inflammation remains an important task to reduce the risk of severe ocular damage.

## ▪ 2456
## Medical Management of paediatric uveitis

*HERBORT CP (1, 2), BOUCHENAKI N (1)*
*(1) Inflammatory Eye Diseases, La Source Eye Centre, Lausanne*
*(2) University of Lausanne, Lausanne*

**Purpose** To evaluate the indications and the contribution of systemic corticosteroids and immunosuppresive therapy in chidhood uveitis.

**Methods** A retrospective case series of children with a chronic or recurrent uveitis seen at the UveitisClinic at la Source Eye Centre between 1995 and 2002 who were treated systemically either with corticosteroids and/or with imunosupressive treatment. Indications, efficacy and side effects were analysed.

**Results** From 1995 to 2002, 79 children with uveitis were seen. Thirty-six (45.5%) received systemic therapy. Among them, sixteen (44.4%) received systemic corticosteroid therapy alone (pars planitis (6), toxoplasmosis (2, with antibiotics), juvenile idiopathic arthritis (2), Behcet's disease (1), inflammatory choriocapillaropathy (1), V-K-H disease (1), undefined diagnosis (3). 20 patients (55.6%) were under systemic immunosuppressive therapy and 14 patients had enough data to be included in the analysis ( juvenile idiopathic arthritis (6 patients), pars planitis (4), Behcet's disease (2), sarcoidosis (3), undetermined (5).The indications to add an other immunosuppressant were the severity of the inflammation (17 patients) and/or the need for a steroid sparing effect (12). Azathioprine was added in 13 patients, methotrexate in 8, cyclosporine in 7, etanercept in 1. After introduction of immunosuppressive therapy disease was controlled in all patients with sufficient follow-up data (n=11) and all were able to reduce their prednisone dose. One patient had to discontinue azathioprine because of liver toxicity.

**Conclusion** A relatively large proportion of childhood uveitis needed systemic corticosteroids and immunosuppressive therapy which was well tolerated and is safe as long as blood and liver tests are performed regularly.

## ▪ 2457
## Surgical Management of paediatric Uveitis

*BODAGHI B*
*Ophthalmology, Paris*

**Purpose** Secondary cataract, glaucoma and band keratopathy are the main complications of pediatric uveitis. Progress in cataract surgery has been performed during the last 10 years in children with juvenile idiopathic arthritis-associated uveitis. This topic remains highly controversial. Intraocular lens implantation has been proposed after posterior capsulorhexis and anterior vitrectomy in different clinical situations. However, the young age of children less than 5 years and the pauciarticular form of JIA seem to be high-risk situations, especially in bilateral forms of the disease and IOL implantation should be performed cautiously. In all clinical situations, IOL implantation may not be proposed without concomitant and aggressive topical and systemic corticosteroids or immunosuppressors in order to control ocular inflammation, not only before surgery, but also during years after surgery. On the other hand use of ocular lenses is difficult in the face of major band keratopathy. Glaucoma surgery is even more complicated in these cases. Drainage tubes may be proposed in challenging cases, but final visual prognosis remains poor when control of intraocular pressure can not be achieved with medical strategies. Screening for secondary glaucoma is a major point in these children and anti-inflammatory strategies should be adapted to clinical situations and flare values evaluated with laser flare photometry in order to prevent overtreatment.

■ 2461

## Morphologic and volumetric studies of the human Meibomian glands

*BRON AJ (1), KOZAK I (2, 3), KLUCHOVA D (4), KUCHAROVA K (5), MARSALA M (5), TIFFANY JM (1)*
*(1) Nuffield Laboratory of Ophthalmology, University of Oxford, Oxford*
*(2) Shiley Eye Center, University of California, San Diego, La Jolla, CA*
*(3) Dept of Ophthalmology, University of P J Safarik, Kosice*
*(4) Dept. of Anatomy, University of P J Safarik, Kosice*
*(5) Anesthesiology Research Laboratory, University of California, San Diego, La Jolla, CA*

**Purpose** A key feature of Meibomian gland dysfunction (MGD) is the loss of orifice landmarks at the lid margin. To investigate the normal anatomy we have examined the structure of human Meibomian glands (MG) with attention to volume and terminal duct morphology.

**Methods** Human, macroscopically normal lower lid samples were obtained at autopsy from 5 men and 4 women with a mean age of 63.1 ± 7.67 years (range 54-81 years). Tissues were fixed, embedded in paraffin and serial, transverse sections cut at 5 µm to include the full length of the glands. Sections were stained with H and E, van Gieson's and Masson blue. Slides were viewed with a Leica DMLB microscope and images captured with an Olympus digital camera with ImagePro 4.5 software and StagePro, for Z-stage motion (Media Cybernetics, Silver Spring, MD, U.S.A). MG volume reconstruction employed a 3-D constructor programme (Media Cybernetics).

**Results** Acini feed via small ductules into a main duct. A connective tissue sheath surrounds each terminal duct at the level of each orifice and deep to this, small acini are arranged in a circular, florette pattern. Average MG lengths in the nasal, central and temporal region were: 1.551 ± 0.43, 1.654 ± 0.47 and 1.594 ± 0.57 mm and surface areas were 0.029 ± 0.03, 0.033 ± 0.01 and 0.056 ± 0.03 mm2 respectively. Average MG volumes in the nasal, central and temporal regions were 0.054 ± 00.4, 0.056 ± 0.03, 0.053 ± 0.03 mm3.

**Conclusion** The concentric structures seen with the slit-lamp around normal Meibomian orifices are due to the presence of a connective tissue sheath and a circular arrangement of acini around the terminal duct. We predict that a disturbance of this anatomy causes the altered lid margin changes of MGD.

■ 2462

## Ultrastructural anatomy of M-cells, germinal centers and high endothelial venules in CALT follicles of the rabbit

*KNOP E (1), KNOP N (2)*
*(1) Research Lab., Dept. of Ophthalmology, Charité, Berlin*
*(2) Dept. of Cell Biology in Anatomy, Medical School Hannover, Hannover*

**Purpose** Conjunctiva-associated lymphoid tissue (CALT) is a part of the eye-associated lymphoid tissue (EALT) at the ocular surface. Its ultrastructure is largely unknown.

**Methods** Flat whole-mount conjunctival tissues (42) from 21 young adult rabbits were investigated native in reflected light, and further stained and cleared (6), in paraffin histology (6), scanning electron microscopy (SEM, 4) and transmission electron microscopy (TEM, 4).

**Results** Secondary lymphoid follicles accumulated into a dense group nasally towards the lacrimal punctum of the lower lid. High endothelial venules (HEV) with typical ultrastructure occurred in the parafollicular zone. The bright germinal center (GC) contained lymphoblasts, follicular dendritic cells, apoptotic cells and tingible body macrophages. The follicle-associated epithelium (FAE) was devoid of goblet cells and contained groups of lymphoid cells. TEM showed them to be located in cytoplasmic pockets of superficial electron lucent with a thin cytoplasmic luminal lining that contained a fine filament meshwork and numerous endocytotic vesicles. The M-cells were sitting between and on top of the ordinary dense epithelial cells located basally and forming pillar-like structures. In SEM, the surface cells were very large, had a polygonal outline and covered cavernous spaces.

**Conclusion** The rabbit has a CALT with typical follicular morphology including HEV for regulated lymphocyte migration and epithelial cells with ultrastructural characteristics of M-cells that allow antigen transport as indicated by the GC-reaction. Their arrangement on top and between epithelial pillar cells may reflect a special structural requirement of the multi-layered CALT FAE.

■ 2463

## Human corneal epithelial cell migration and expression of laminin-5 by insulin-like growth factor-1(IGF-1) in cultured human corneal epithelial cell

*LEE HK, KIM EK, LEE JH, HAN SH*
*Ophthalmology Yonsei University College of Medicine, Seoul*

**Purpose** We investigated whether insulin-like growth factor-1 (IGF-1) could stimulate human corneal epithelial cell (hCEC) migration and provide the evidence that IGF-1 involves in laminin-5 induction. Also, we investigate whether IGF-1 can change the expression of Ln-5 associated integrins during the in vitro hCEC migration.

**Methods** The hCEC migration by IGF-1 and laminin-5 (Ln-5) was determined by wound break assay and chemoattractant assay. The expression of laminin-5 and fibronectin (Fn) by IGF-1 was determined with western immunoblot. The expression of integrin β1 and α3 by IGF-1 was determined by confocal microscopy and western immunoblot. Pretreatment of hCEC with αIR3, LY 294002, PD 98059 was used for migration inhibition assay.

**Results** Cell migration was increased by IGF-1 and Ln-5. IGF-1 enhanced the production of laminin-5 by dose and time dependent manner. But Fn production was not affected by IGF-1. IGF-1 induced Ln-5 production was prevented with the pretreatment of αIR3 and LY 294002. Integrin β1 production was increased by IGF-1. The Integrin β1 production was not influenced by Ln-5.

**Conclusion** We have identified IGF-1 induced HCEC migration via laminin-5 and β1 integrin production. Laminin-5 and β1 integrin production was mediated via activation of PI3-K/AKT pathway, but the MEK-ERK pathway was not involved. The induction of laminin-5 and β1 integrin by IGF-1 was not found to be on cross-talk, and seems to be produced independently. Further refinement of signal control to produce matrix protein by cytokine and the specific activation of MAP kinase by IGF-1 should be needed.

■ 2464

## Identification of c-Kit expressing cells in the Ciliary Muscle of Cebus apella

*VERONESE RODRIGUES ML (1), PAULA JS (1), SOUZA ESMV (1), SOUZA NV (1), GARCIA SB (2)*
*(1) Ophthalmology, Othorhinolaringology and Head and Neck Surgery - Medical School of Ribeirão Preto - University of São Paulo, Brasil, Ribeirão Preto*
*(2) Department of Pathology - Medical School of Ribeirão Preto - University of São Paulo, Brasil, Ribeirão Preto*

**Purpose** Interstitial cells of Cajal (ICC) were identified in the gastrointestinal tract of several species, with close relation with the autonomic nervous system. Since it was recognized that ICC express the gene product of c-kit, we performed immunohistochemistry to Kit protein on ciliary muscle specimens of monkeys' eyes.

**Methods** Eight eyes from four adult male new world monkeys (Cebus apella - from Capuccin Monkeys Procreation Nucleus of the School of Odontology of Araçatuba, UNESP, Brasil) were studied. Sections 4 µm thick were prepared from two paraffin blocks which were taken from segments of horizontal half of the eyes. One was used for HE staining and the other was used for immunohistochemistry. After blocking endogenous peroxidase activity and nonspecific protein binding, 1:100 dilution of mouse monoclonal antibody against c-Kit human oncoprotein was applied to tissues. Antigen-antibody reaction was visualized using the avidin-biotinylated horseradish peroxidase complex in each slide.

**Results** It was observed some groups of fusiform Kit expressing cells, located amongst muscular bundles and in the vascular walls of the ciliary muscle. Other pigment cells and mast cells were also observed.

**Conclusion** Kit expressing cells observed in the ciliary muscle of Cebus apella with no structural similarity to melanocytes or mast cells could indicate the presence of ICC analogous cells in this tissue.

## ▪ 2465
## Myofibrillar ultrastructure of the human extraocular muscles

*CARLSSON E (1), PEDROSA DOMELLOF F (1, 2)*
*(1) IMB, Anatomy, Umeå University, Umeå*
*(2) Dept of Clinical Sciences, Ophthalmology, Umeå University, Umeå*

**Purpose** To determine the myofibrillar ultrastructure of the fibers of the human extraocular muscles (EOM) and to correlate the ultrastructural appearance of the M-bands and Z-discs with the distribution patterns of the corresponding myofibrillar proteins.

**Methods** Longitudinal sections of adult human EOMs were studied with transmission electron microscopy, cryoultramicrotomy and immunocytochemistry using antibodies specific against the M-band and Z-disc proteins and different myosin heavy chains (MyHC).

**Results** At the ultrastructural level no typical M-band patterns were observed in the majority of the fibers studied and the Z-discs were clearly wider than those of skeletal muscle fibers. The M-band composition varied along the length of the human EOMs. Myomesin was excluded from practically all fibers in the mid- and proximal portion of the rectus inferior but present in the fibers at the distal part of the same sample. EH-myomesin was present in the fibers lacking myomesin, but it was also co-expressed in fibers containing myomesin. M-protein was present only in subsets of the fibers containing MyHCextraocular but lacking MyHCslow or fast 2A.

**Conclusion** The myofibrillar ultrastructure of the human EOMs differed from that reported for limb muscles with respect to the M-band and Z-disc appearance. The width of the Z-discs in limb muscle fibers correlates to different fiber types, slow twitch fibers having the wider Z-discs. Work is underway to correlate the ultrastructural appearance of the Z-discs with their protein composition in the EOMs. The novel M-band protein profiles of the EOM fibers and their ultrastructural characteristics confirm the unique character and functional complexity of these muscles.

## ▪ 2466 / 264
## Mediation of Ghrelin's relaxing effect on iris sphincter and dilator muscles

*ROCHA DE SOUSA A (1, 2), SARAIVA J (1), FARIA PA (2), FALCÃO REIS F (2), LEITE-MOREIRA A (1)*
*(1) Physiology, Fac Medicine University of Porto, Porto*
*(2) Ophthalmology S Joao Hospital, Porto*

**Purpose** Ghrelin is a 28 aa peptide with important functions in smooth, cardiac and skeletal muscles. Some of its muscular effects seam mediated by a receptor other than GHRS–1a. The aim of this study was to investigate the mediation of its ocular effects by the GHSR–1a receptor in rat and rabbit iris sphincter or dilator muscle.

**Methods** Rabbit, Wistar rat iris sphincter muscles and rabbit iris dilator muscle were dissected, mounted on a vertical organ (modified Krebs–Ringer; 1.8 mM Ca2+; 35ºC) attached to a force transducer and precontracted. The effects of 1–5,Ghrelin (Gr, 10e–9– 6*10e–5M) on rabbit sphincter (n=7), dilator (n=6) and rat sphincter(n=6) muscle were evaluated. On rabbit sphincter that effect was also tested in the presence of i) L-NA (10e-4M; n=14); ii) indo (10e-5M; n=14); iii) DLysGHRP6 (10e-4M; n=12). Finally, the effect of 1–5,des–octanoil Ghrelin (DGhr; 10e–9– 6*10e–5M) on rabbit sphincter (n=7), dilator (n=6) and rat sphincter (n=6) muscles were evaluated.

**Results** Ghrelin promoted a concentration-dependent relaxation of the rabbit sphincter (34.1±12.1%), dilator (25.8±5.0%) and rat sphincter (63.3±7.1%) muscles maximal at 6*10e-5 M. On rabbit sphincter there was a trend for L-NA to exacerbate (58.0±10.5%) and for Indo to attenuate (20.3±12.1%) ghrelin's relaxing effect. DLysGHRP6 does not inhibit that relaxing effect (118.1±21.1%). DGr promoted also a similar relaxation of rabbit (43.4±5.2 %) and rat (60.5±42.2 %) sphincter. However in the rabbit dilator DGr did not have effect (–3.1±12.1 %).

**Conclusion** Ghrelin relaxation is mediated by GHSR1a in iris dilator but not in iris sphincter muscles.

## ▪ 2467 / 265
## The ß-defensin HBD2 induces specific patterns of Ca2+ responses in conjunctiva cells (IOBA-NHC) associated with activation of L-type channels

*MERGLER SM (1), SCHLORF TS (2), RECKER KR (2), PLEYER U (1), DIEBOLD YD (3), PAULSEN FP (2)*
*(1) Dept. Ophtalmology, Charité - University Medicine Berlin, Berlin*
*(2) Anatomy and Cell Biology, Martin-Luther-University Halle-Wittenberg, Halle*
*(3) Applied Ophthalmobiology, University of Valladolid, Valladolid, Valladolid*

**Purpose** Corneal and conjunctival epithelial cells are constanly exposed to potential pathogenic agents such as infectious organisms. Recently, antimicrobial peptides such as ß-defensins (human ß-defensins 1-3, HBD1-3) that are induced after microbial contact have been shown to be effective in the protection of the ocular surface against distinct bacteria. However, the expression and regulation of HBD1-3 at epithelial surfaces are not clearly known. Interestingly, Ca2+ triggers the expression of HBD2 in human keratinocytes. Aim: Characterization of the effects of HBD2 on intracellular Ca2+ concentration ([Ca2+]i) in a permanent conjunctiva cell line (IOBA-NHC).

**Methods** The effects of recombinant HBD2 were determined in fura2-loaded IOBA-NHC cells with a fluorescence video imaging system.

**Results** Ca2+ responses in IOBA-NHC cells were clearly detectable in a Na- and K-free solution. Specifically, extracellular application of HBD2 (100 ng/ml) increased [Ca2+]i to a peak level of 109 ± 1 % of control (set to 100 %) followed by a recovery to the baseline after 5 min (± SEM; n = 5). In the presence of the L-type channel blocker nifedipine (5 µM), [Ca2+]i significantly decreased to 80 ± 4 % of control (n = 4; $p < 0.0001$).

**Conclusion** Conjunctiva cells display Ca2+ patterns in responses to HBD2 depending on voltage-dependent L-type Ca2+ channel activity. This could play an important role in the expression and regulation of the ß-defensin HBD2 in the conjunctiva. Further investigations are currently being performed to better understand the effect mechanisms of HBD2.Supported by Wilhelm Roux program grant FKZ 09/16, Sicca-research-support and DFG PL/150-14 (in part)

■ 3111

## Ocular pathology in Turner's syndrome

*MULAK M (1), MISIUK-HOJLO M (1), SZALINSKI M (1), WIKIERA B (2)*
*(1) Ophthalmology Department of Medical University, Wroclaw*
*(2) Pediatric Endocrinology of Medical University, Wroclaw*

**Purpose** Turner's syndrome (TS) is a common chromosomal abnormality usually accompanied by characteristic changes in phenotype of the patients. It is also associated with more frequent ophthalmic morbidity. The goal of the study was to investigate the prevalence and severity of the ophthalmic problems in patients with TS.

**Methods** 60 girls with TS aged 4-23 (mean 15.46) were involved into the study. They were diagnosed in the Department of Pediatric Endocrinology and Diabetology. Full opthalmic examination was performed in the Department of Ophthalmology in all of them. Most of the patients were examined by oculist for the first time. The diagnosis of TS was established on a detailed karyotype analysis.

**Results** It revealed that 60% of the patients had 45,X monosomy, 36.3% different mosaic pattern and 3,3% structural chromosomal aberration. Different eye diseases were confirmed in 35% of the whole group. 35% of the patients suffered from an inappropriate visual acuity, 20% from a convergent squint, 1.7% from divergent squint and a choroidal naevus, 5% from a defective color vision, 1.7% from an ocular hypertension and an increased corneal thickness on pachymetry, 3.3% from drusen. In 1.7% of the patients anterior segment defect occured. It was malformation of an iris. On ophthalmoscopy an optic nerve disc oedema was found in one patient. The girl was referred to MR examination. Tortuous vessels of the ocular fundi were present in another patient. An epicantus was found in 3.3% girls wit TS. There was no correlation between the ocular findings and the pattern of TS karyotype.

**Conclusion** Our study confirms that different ocular problems occured in TS frequenly. A routine ophthalmologic examination is warranted early in TS patients to diagnose and treat confirmed abnormalities.

■ 3112

## Ocular manifestations in liver transplant recipients with familial amyloid polyneuropathy

*SANDGREN OJP (1), KJELLGREN D (1), SLIHR OB (2)*
*(1) Department of Clinical Sciences/Ophthalmology, University of Umeå, Umeå*
*(2) Department of Medicine, University of Umeå, Umeå*

**Purpose** As a result of the ocular synthesis of TTR, the liver transplanted (LT) familial amyloidotic polyneuropathy (FAP) -patients are still at risk for developing ocular complications, which also previously has been reported. The aim of the present study is to evaluate the postoperative ocular involvement of Swedish liver transplant recipients with FAP.

**Methods** Out of 52 still living transplant recipients, 48 had been followed-up in Umeå. Routine ophthalmological examinations were performed with particular attention regarding amyloid deposition in the anterior segment and the vitreous body. Medical records were scrutinised for information regarding duration of disease and neurological impairment at the time for LT. Thirty-six of the patients had underwent a preoperative ophthalmological examination. The diagnosis was secured in all cases by the finding of amyloid deposits in biopsy specimens and positive genetic testing for ATTR Val30Met mutation.

**Results** Five patients (10 %) developed vitrious opacities within the post-LT observation period of 133 months. The first developed opacities 40 months after transplantation and 8 years after onset of disease. Seven patients (15 %) developed glaucoma of which the first was observed 18 months after the procedure and 6.5 years after onset of the disease. Fifteen patients (31 %) developed deposits on the lens. Besides, scalloped pupillary margins and/or retinal changes were noted in 11 patients (23 %).

**Conclusion** The prevalence of eye complications increase with time after transplantation, and a regularly follow-up is necessary, especially to disclose the development of glaucoma, a complication the patients themselves not normally are aware of.

■ 3113

## The comparison of different refractive error cut-off points to define myopia

*SAW SM (1, 2, 3), GAZZARD G (4), LUO HD (2), FOSTER PJ, SHANKAR A (1), TAN DTH (2, 3, 5)*
*(1) Community, Occupational and Family Medicine, National University of Singapore, Singapore*
*(2) Singapore Eye Research Institute, Singapore*
*(3) Ophthalmology, National University of Singapore, Singapore*
*(4) Institute of Ophthalmology, United Kingdom*
*(5) Singapore National Eye Centre, Singapore*

**Purpose** To determine an optimal spherical equivalent (SE) cut-off to define myopia.

**Methods** The baseline data from 1334 Chinese schoolchildren aged 7 to 9 years from 3 schools were enrolled in the Singapore Cohort Study of the Risk Factors for Myopia (SCORM). Uncorrected distance logMAR visual acuity measures were made in the right and left eyes. Cycloplegia was induced using 3 drops of 1% cyclopentolate administered 5 minutes apart and table-mounted autorefraction measures were performed. Commonly used SE cut-off points for myopia [-0.25 Diopters (D), -0.5D, -0.75D, -1.0D] were evaluated.

**Results** Using different definitions of myopia, the prevalence rates of myopia in the SCORM study varied from 45.8% (SE at least -0.25 D) to 30.7% (SE at least -1.0 D). A definition of myopia of spherical equivalent of at least -0.75 D had a sensitivity and specificity of 90.2% (95% CI, 87.4 to 92.9) and 94.7% (95% CI, 93.2 to 96.2), respectively to predict visual impairment defined as uncorrected logMAR visual acuity > 0.3 (either eye). The next best definition of -0.5 D had a higher sensitivity (90.2), but lower specificity (88.8). The definitions of -0.25 D and -1.0 D have unacceptably low specificities (81.2%) and sensitivities (83.7%), respectively.

**Conclusion** The cut-off point of -0.75 D in spherical equivalent refraction is appropriate for the definition of myopia. The definition of -0.5 D may also be used.

■ 3114

## Refractive error in urban and rural adult Chinese in Beijing

*JONAS JB (1), XU L (2), LI J (2), CUI T (2), HU A (2), FAN G (2), ZHANG R (2), YANG H (2), SUN B (2)*
*(1) Department of Ophthalmology, Faculty of Clinical Medicine Mannheim, University of Heidelberg, Mannheim, Germany, Mannheim*
*(2) Beijing Institute of Ophthalmology, Tongren Hospital, Beijing, China, Beijing*

**Purpose** Purpose. To evaluate the refractive error and its demographic associations in an urban and rural population in Northern China.

**Methods** Methods. The Beijing Eye Study is a population-based cohort study in Northern China including 4439 subjects out of 5324 subjects asked to participate with an age of 40+ years. It was divided into a rural part (44.1% of subjects) and an urban part (55.9% of subjects). Main outcome measure was refractive error.

**Results** Results. Mean refractive error measured −0.33 ± 2.22 diopters (range, -20.88 diopters to +7.88 diopters). Myopia of > -0,50 D, −1.0 D, >-6.0 D, and > -8D, respectively, occurred in 22.9%, 16.9%, 2.6% and 1.5% of the subjects, respectively. In a multiple regression analysis, myopic refractive error was significantly associated with younger age ($p<0.001$), urban region versus rural region ($p=0.024$), higher educational background ($p=0.013$), and higher degree of nuclear cataract ($p=0.048$). Prevalence of high myopia defined as a myopic refractive error of more than −8 diopters, was significantly associated with age ($p<0.001$), female gender ($p=0.020$), urban region ($p=0.023$), and lower best corrected visual acuity ($p<0.001$). In a multiple regression analysis, astigmatism increased significantly with age ($p<0.001$), lower uncorrected visual acuity ($p<0.001$) and lower best corrected visual acuity ($p<0.001$), females versus men ($p=0.001$), rural area versus urban area ($p<0.001$), and the degree of nuclear cataract ($p=0.047$).

**Conclusion** Conclusions. In the Northern Chinese population, aged 40 years or older, mean refractive error is −0.33 ± 2.22 diopters. Myopic refractive error was associated with lower age, urban region, higher educational background, and degree of nuclear cataract.

## ▪ 3115 / 364
### To ascertain the prevalence of poor vision which exists in boys at a prestigious school in a developing country (Trinidad)

*DWARIKA D (1), BHOLA RM (2), SEEMONGAL DASS R (3), EMAMALIE S (2)*
*(1) Birmingham and Midland Eye Centre, Birmingham*
*(2) Academic Unit of Ophthalmology and Orthoptics, The University of Sheffield, Royal Hallamshire Hospital, Sheffield, Sheffield*
*(3) The Eric Williams Medical Sciences Complex, Trinidad*

**Purpose** This study aims to ascertain the prevalence of poor vision which exists in boys in a prestigious school in a developing country (Trinidad).

**Methods** The study included two parts: a short observer administered questionnaire and an assessment of visual acuity. Monocular visual acuities were assessed using imperial standard Snellen charts from a distance of 20 feet. Visual acuities were performed with glasses, unaided, and pinhole. Glasses were neutralized and results recorded.

**Results** A total of 334 students ranging in age from 11 to 18 were tested. Seventy students failed to achieve the required visual acuity in one or both eyes (20.96%). One hundred and seventy students never had an eye test (51%). Of the one hundred and sixty four who did have an eye test, 46 had a refractive error needing correction. Eighteen were myopic and the remaining 28 had myopic astigmatism.

**Conclusion** More that 50% never had a formal eye examination and 1 in 5 students failed to achieve a visual acuity of 20/25. This result emphasizes the need for formal vision screening in schools in Trinidad.

## ▪ 3116 / 365
### Ocular complications and evaluation of quality of life in patients after Stevens-Johnson and Lyell syndromes

*GUEUDRY J (1), BINAGHI M (2), SOUBRANE G (2), ROUJEAU J-C (3), BRASSEUR G (1), MURAINE M (1)*
*(1) Ophthalmology, Rouen*
*(2) Ophthalmology, Créteil*
*(3) Dermatology, Créteil*

**Purpose** Toxic epidermal necrolysis (TEN) and Stevens-Johnson syndrome (SJS) are rare, life-threatening, drug-induced mucocutaneous reactions. The purpose of these present report is to describe the ocular sequelae and to quantify their impact on quality of life.

**Methods** We retrospectively reviewed the medical records of patients with Stevens-Johnson and Lyell syndromes seen between 1994 and 2002 at the university hospital of Creteil. Patients underwent a recent ophthalmologic examination and completed the OSDI (ocular surface disease index). It measure dry eye disease severity and effect on vision related function. It is assessed on a scale of 0 to 100, with higher scores representing greater disability.

**Results** One hundred and sixty five patients were identified. Thirty two patients (19%) died during their hospitalisation, 17 patients (10%) died secondarily, 11 patients were excluded because of their poor compliance, finally 42 patients were non completers.OSDI of 66 patients were analysed. Mean age was 47 years. Mean follow up was 7 years. Sixty five percent of patients had dry eye symptoms, mean OSDI score was 31. Fifty one patients underwent clinical examinations. Best corrected visual acuity was 6/10 or better in 32/44 (96%) patients. Lid abnormalities were observed in 8/51 (16%) patients, conjunctival abnormalities in 16/51 (29%), corneal complications in 15/51 (29%) and sicca eyes syndrome in 30/51(59%).

**Conclusion** We report here the most important series of patients with SJS syndrome and TEN. In contrast with other smallest and rare series, only few patients (7%) have severe ocular complications with very poor quality of life.

## ▪ 3117 / 366
### Missed metal Foreign Bodies

*SKINNER KC, CHRONI FC, MERRICK CD, GREGORY PT*
*Conquest hospital, St-Leonards-on-Sea*

**Purpose** To emphasise the importance of history taking where a possible metal foreign body is suspected. The importance of x rays in these case and their suitability as a screening process.

**Methods** Case Series

**Results** Three patients all presenting with metal foreign bodies detected by means other then direct observation.One: attended A and E in 2000. History, hammering metal nail. A and E SHO noticed possible metal foreign body embedded in sclera. Patient seen by on call eye SHO no foreign body noted but large sub conjunctival haemorrhage. Patient subsequently DNA all follow up appointments. 5 years later attended for MRI scan due to possible fits. X ray of orbits done as patient reported past history metal FB. Xray revealed presence metal in anterior aspect of globe.Two: attended A and E in 2005 after hammering nail. Three days later developed very painful red eye. A and E SHO could not see any FB but could see small scar on cornea. Xray done and metal FB noted in anterior segement. Eye SHO saw patient and noted large FB embedded in temporal aspect of iris.Three: attended eye clinic for cataract. Staff grade saw patient and noticed abnormal shaped optic disc. Seen by consultant who noted possible metal FB embedded in optic disc, in fact later old notes revealed probably been there since 1976.

**Conclusion** IOFB of metal composition leads to long term complications such as siderosis etc. It is therefore very important not to miss these when seeing a patient. One indicator to the high possibility of IOFB is the history that the patient gives, e.g hammering metal on metal. In these cases a very thorough examination must be performed and if no FB found then Xray considered. As MRI is becoming more used it is especially important not to miss metal IOFB.

## ▪ 3118 / 367
### OPHTEC Iris Reconstruction Lens in Ocular Trauma

*KULJACA Z (1), IGNJATOVIC Z (1), DZAKOVIC N (1), PAVLOVIC S (2, 1)*
*(1) Milos Clinic, Belgrade*
*(2) University of Giessen,Dpt. of Ophthalmology, Giessen*

**Purpose** To see how penetrating eye injury in young patient with visual and cosmetic sequels can be repaired with satisfactory results concerning visual acuity as well as good looking.

**Methods** Young female, aged 17, presented with penetrating injury of her left eye that was primary repaired two weeks ago. She was injured with glass in car crash and had intumescent traumatic cataract, vitreous hemorrhage and complete avulsion of the iris. In the first step pp lensectomy and vitrecomy was performed. Due to peripheral retinal tears silicon oil tamponade was necessary. After three months in second step surgery silicon oil was removed and Ophtec aniridia IOL was placed in sulcus using the remaining parts of the anterior capsule.

**Results** After four months the visual acuity was 0,7 and the retina is attached. There is no glare or photophobia and both eyes look the same in everyday life.

**Conclusion** After major and disfiguring ocular trauma it is important to repair both vision and appearance whenever possible in order to fully satisfy the patient.

## ▪ 3121
## After-Cataract Dilemmas

*VRENSEN G*
*Ophthalmology, Univ. Medical Center, Leiden*

**Purpose** PCO is caused by unrestrained proliferation of LECs left in the bag after ECCE. LECs migrate to the posterior capsule causing wrinkles and fibrotic plaques or differentiate into lens fibres forming the ring of Soemmering. Escaping fibres form Elschnig's pearls. Plaques and pearls obstruct light and capsulotomy is needed to restore vision.Efforts to overcome LEC proliferation and migration by cleaning the capsule, by mitotic inhibition and killing of LECs, by creating a barrier between the peripheral and central PC with special IOL designs or by removal of the central PC have reduced the prevalence of PCO but it is still at a disappointing level of 10-20%. Dilemmas: careful cleaning considerably extends the ECCC procedure, and mitotic inhibition or cell killing should be 100% effective in a short per-operative period and should not affect other anterior chamber tissues. Even in the 80ties ECCE and IOL implantation were uncomplicated in 40-50% of cases. A probable reason for this is the formation of the ring of Soemmering consisting of a monolayer of slowly proliferating LECs and a core of lens fibres. As long as Soemmering's ring remains closed nothing seems to happen. It was shown in vitro that lens fibres left after ECCE reduce the proliferation of LECs. The 'bag-in-the-lens' implantation of IOLs (Tassignon et al., JCRS 2002), in which a closed ring is intrinsic to the procedure, proves this point. Up till now no PCO has been observed after this procedure.The 'bag-in-the-lens' procedure seems an excellent solution for PCO prevention but its surgery is rather complicated. Alternatively we could intentionally leave a rim of equatorial lens fibres during ECCC or try to find the lens fibre factors controlling the proliferation and differentiation in the intact lens.

## ▪ 3122
## Clinical aspects of Posterior Capsular Opacification

*TETZ M*
*Berlin*

**ABSTRACT NOT PROVIDED**

## ▪ 3123
## Are the lens epithelial cells the capsular bag's enemy or ally?

*TASSIGNON MJ*
*Department of Ophthalmology, Antwerp*

**Purpose** The last decade, the lens epithelial cells (LECs) have received full responsibility for the occurrence of PCO after cataract surgery. We would like to prove that this is only partially true.

**Methods** - Clinical evaluation of the capsular bag of 300 eyes implanted with the bag-in-the-lens were evaluated.- Capsular bags equipped with a bag-in-the-lens IOL were put in culture and compared to capsular bags equipped with a lens-in-the-bag IOL.

**Results** The capsular bags of 123 eyes could be monitored on the presence of Elschnig pearls, fibrosis, wrinkles and Soemerring.The response of the LECs on TGF-β was much lower for the bag-in-the-lens capsules compared to the lens-in-the-bag capsules.

**Conclusion** The bag-in-the-lens implantation presents the advantage to have full control over the LEC transformation by allowing only LEC proliferation. The physiological conditions for the maintenance of the capsular bag are better preserved using the bag-in-the-lens implantation technique. This is a major advantage.

## ▪ 3124
## Surgical options for PCO prevention

*BARRAQUER RI*
*J. Barraquer Chair of Eye Research - UAB, Barcelona*

**Purpose** To review the current status of posterior capsule opacification (PCO) prevention strategies and surgical options.

**Methods** Literature review and meta-analysis of the factors influencing PCO. Long-term retrospective study of personal series comparing rates of PCO with and without lens epithelial cell aspiration (LECA). Retrospective study of the incidence of retinal complications of planned posterior capsulorhexis (PPC) versus Nd:YAG capsulotomy (YAG).

**Results** A number of pathological conditions, surgical techniques, IOL designs and materials, and pharmacological treatments have been described to influence the rate of PCO. With IOLs of classical design and materials (PMMA, silicone), we found a significant reduction in PCO at over 3 years of follow-up, from ECCE and no LECA (61.8%), to phacoemulsification (PE) without LECA (36.6%), to PE+LECA (17.2%, $p<0.01$). In another study, retinal detachment (RD) occurred in 1.05% of cases after PPC versus 2.14% after YAG (OR=2.05 but p=0.31). Both groups were significantly worse than the intact capsule group (IC, 0.24% RD) and significantly better than the unplanned capsular rupture group (8.12% RD). Rates of cystoid macular edema and new retinal tears were non significantly different between IC, PPC and YAG.

**Conclusion** PCO remains the most frequent postoperative complication after cataract surgery. LECA is an effective surgical method for the reduction of PCO incidence. Further studies are required to evaluate its possible additivity with the documented beneficial effect of the newer IOL designs and materials. PPC is a viable option in the younger patients with high risk of PCO, appearing to be at least as safe as YAG capsulotomy.

■ 3125

## Intra- & post-operative abrasion of the lens epithelial cells by the IOL

*GALAND A*
*Centre d'Ophtalmologie, Liège*

**Purpose** To get partial or total elimination of the lens epithelial cells in order to reduce the rate of posterior capsular opacification.

**Methods** I observed by slit lamp examinations that eyes with intraocular lenses implants smaller than the capsular bag had sometimes no posterior capsule opacification (PCO), up to ten years postoperatively.In those eyes, a rotation of the implant within the capsular bag, after the surgery, was either documented or supposed. So, I assumed that a relative freedom of the implant can result in abrasion of the lens epithelial cells. "Sine Tensione" intraocular lenses allow some, limited, pseudo-accommodation by anterior shifting. By intra and post-operative rotation of such implants (AccoRing), we hope a reduction of the PCO rate.

**Results** After a one year follow-up, eyes which received the implant, with intraoperative rotational manoeuvre, present a lower PCO rate than eyes with conventional implantation.However the respective role of the implant design and of the surgical method are difficullt to assess.Data and their discussion will be bring at the SIS PCO.

**Conclusion** Intra- and post- operative implant rotation could be a way to reduce PCO. Textured haptics are suggested to improve this possible mechanism of abrasion of the lens epithelial cells.

■ 3126

## A human capsular bag model for PCO evaluation after IOL implantation - prognostic power?

*LIEKFELD A*
*Charité, Ophthalmology, Berlin*

**Purpose** To evaluate the transferability of a human capsular bag model to clinical situations concerning the development of posterior capsule opacification after intraocular lens (IOL) implantation.

**Methods** 120 intraocular lenses were compared in pairs in the capsular bag model. The behaviour of the lens epithelial cells (LEC) was documented in terms of the time needed to form a complete monolayer on the posterior capsule. The results were compared to data from the literature and from own clinical studies.

**Results** Depending on the type of IOL the LEC growing was significantly different in speed. A LEC monolayer was formed between day 8 at the earliest and more than 60 days at the latest.

**Conclusion** The human capsular bag model employed allows short-term evaluation of secondary cataract formation for different IOLs. This model's correlation with clinical results is good.

## ▪ 3131
## Basic mechanisms of steroids (triamcinolone acetonide)

*JOUSSEN Antonia M*
*Department of Vitreoretinal Surgery, Center of Ophthalmology, University of Cologne, Cologne*

**Purpose** To explain the role of steroids in reducing blood barrier breakdown.

**Results** This overview presents the mechanisms resulting in macular edema: endothelial cell damage, junctional complexes, and cell permeability. Steroids interact with vascular permeability in different ways including inhibition of prostaglandinmediated pathways and direct interaction with cytokines. Furthermore they interact directly with the expression of junctional molecules. Intravitreal kineticsof steroids are discussed

## ▪ 3132
## Randomized trial on the dosage dependency of low dosage and high dosage of intravitreal trimacinolone

*JONAS JB, SPANDAU UH*
*Department of Ophthalmology, Faculty of Clinical Medicine Mannheim of the University Heidelberg, Mannheim*

**Purpose** To evaluate the effect of different dosages of intravitreal triamcinolone acetonide on diffuse diabetic macular edema.

**Methods** The prospective, randomized, double masked, clinical interventional study included 27 eyes (27 patients) with diffuse diabetic macular edema. They were randomly divided into three study groups receiving an intravitreal injection of filtered triamcinolone acetonide of about 2 mg (n=8 eyes), 5 mg (n=10) or 13 mg (n=9), respectively. Dosage measurement was performed prior to filtration. Mean follow-up was 6.6 ± 2.4 months (3 to 12 months). Main outcome measures were was visual acuity and intraocular pressure.

**Results** Maximal increase in visual acuity was significantly (p=0.046; 95%CI: 0.032 to 2.99; r=0.38) correlated with the dosage of intravitreal triamcinolone acetonide. Additionally, the duration of the effect of intravitreal triamcinolone acetonide increased significantly with the dosage of intravitreal triamcinolone acetonide (r=0.45; p=0.014). Increase in intraocular pressure during follow-up was statistically not significantly associated with the dosage used (p=0.77).

**Conclusion** In patients with diffuse diabetic macular edema receiving intravitreal triamcinolone acetonide, treatment response may last longer and be more pronounced with a dosage of 13 mg than in lower dosages of 5 mg or 2 mg. Triamcinolone acetonide induced increase in intraocular pressure may not markedly be associated with the dosage used.

## ▪ 3133
## Intravitreal steroids associated with laser therapy in the treatment of retinal vascular diseases

*BANDELLO FB*
*Department of Ophthalmology, Udine*

**Purpose** To evaluate the effectiveness of intravitreal injection of triamcinolone acetonide (IVTA) as adjunctive treatment to laser photocoagulation in retinal vascular diseases

**Methods** 53 eyes of 44 patients were enrolled. Three groups were considered. (Group 1) 9 patients, with bilateral proliferative diabetic retinopathy (PDR), in one eye received IVTA (4 mg) before PRP (injected eye) and the other underwent PRP alone (control eye). The main outcome measures were the changes in fluorescein leakage (FL mm2) from new vessels, in central macular thickness (CMT) on optical coherence tomography (OCT), in best corrected visual acuity (BCVA) and the intraocular pressure (IOP) measured at 3, 6 and 9 months. (Group 2) 20 eyes of 20 patients with diffuse CSME were evaluated, 10 eyes randomised to receive one IVTA (4 mg) followed by grid-laser photocoagulation (IVTA + laser eyes) and 10 eyes randomised to IVTA (IVTA-only eyes). Outcome measures were the changes in CMT measured by OCT and in BCVA, at 1 week, 1, 3, and 6 months. (Group 3) 15 eyes of 15 patients with macular edema due to branch retinal vein occlusion received one IVTA (4 mg) followed by laser treatment. Outcome measures were the changes in CMT, measured by OCT, in BCVA and in IOP at 1, 3, 6 months.

**Results** In Group 1 FL and CMT were reduced significantly in the injected eyes with respect to the control eyes. Mean BCVA remained stable and was reduced respectively. In Group 2 the difference between the CTMs and BCVA of IVTA+laser eyes and IVTA-only eyes was significant at the end of follow-up. In Group 3 CMT was reduced and BCVA improved at the end of follow-up with respect to the baseline.

**Conclusion** Combined treatment with IVTA and laser provide benefit in retinal vascular diseases

## ▪ 3134
## Intravitreal triamcinolone acetonide as an additional tool in pars plana vitrectomy for proliferative diabetic retinopathy and other diseases

*KARACORLU M*
*Istanbul Retina Institute, Inc., Istanbul*

**Purpose** The purpose of this presentation is to describe the potential indications, potential complications,and associated techniques for use of intravitreal triamcinolone as a surgical adjunct.

**Methods** Intravitreal triamcinolone acetonide (IVTA) can be used as an intravitreal surgical tool in different type of vitreo-retinal pathologies. Triamcinolone has also been injected at the end of the surgery to control the postoperative inflammation and especially macular edema. It might also diminish the negative effect of panretinal laser photocoagulation during and after the surgery.

**Results** The use of IVTA as an adjunct during vitrectomy surgery for certain diseases is gaining popularity among vitreoretinal surgeons. Small case series have highlighted the usefulness of intraoperative IVTA for visualization of the vitreous body during vitreous removal. Epiretinal membrane removal, posterior hyaloidal separation, removal of preretinal proliferative vitreoretinopathy (PVR), and internal limiting membrane (ILM) peeling are all conditions in which IVTA potentially can enhance visualization during surgery. Unlike ICG, which specifically stains the ILM, triamcinolone appears to "dust" all preretinal structures. This gives the vitreous and other preretinal structures an opaque quality that is inherently easier to identify.

**Conclusion** The use of triamcinolone in vitreoretinal diseases appears promising as both a surgical tool and a therapeutic agent. The demonstration of optimal techniques to limit side effects and complications associated with IVTA will also improve the outcomes with IVTA and improve patients' quality of life.

■ **3135**

## Duration of the effect of intravitreal triamcinolone acetonide

*JONAS JB, SPANDAU UH, DEGENRING RF, KREISSIG I, AKKOYUN I*
*Department of Ophthalmology, Faculty of Clinical Medicine Mannheim of the University Heidelberg, Mannheim*

**Purpose** To evaluate the duration of the effect of intravitreal triamcinolone acetonide on visual acuity in patients with exudative age-related macular degeneration

**Methods** The prospective clinical interventional case-series study included 42 patients (44 eyes) with exudative age-related macular degeneration, who had shown an increase in visual acuity by at least 2 Snellen lines after an intravitreal injection of 25 mg triamcinolone acetonide. Mean follow-up time was 10.4 ± 7.0 months (3.1 months – 31.7 months).

**Results** Within the first week after the injection, visual acuity and intraocular pressure started to increase significantly ($p<0.01$) to reach a plateau-like maximum at 1 – 6 months after the injection. Visual acuity returned to baseline values 8 – 9 months after the injection. Intraocular pressure elevation was statistically ($p>0.15$) independent of the increase in visual acuity.

**Conclusion** In patients with an increase in visual acuity after intravitreal injection of 25 mg triamcinolone acetonide as treatment of exudative age-related macular degeneration, visual acuity and intraocular pressure start to increase with the first 4 weeks after the injection, reach a plateau-like maximum 1- 6 months after injection, and return to baseline values about 8 – 9 months after the injection. It may suggest that triamcinolone may be re-injected about 6 - 9 months after a primary successful injection. Intraocular pressure has to be checked for several months after the injection. The finding that visual acuity increased and decreased in a chronological parallel manner with the steroid induced change in intraocular pressure suggests that the increase in visual acuity was connected with the intraocular presence of triamcinolone acetonide.

■ **3136**

## New techniques for delivery of steroids

*KUPPERMAN L*

**ABSTRACT NOT PROVIDED**

■ **3141**

## Choroidectomy versus choroidectomy associated with brachytherapy

*PRIMAVERA V, IACULLI C, ERCOLE I, DELLE NOCI N*
*Clinica Oculistica Università di Foggia, Foggia*

**Purpose** Transscleral choroidectomy and cyclochoroidectomy are methods aimed at conserving the eye in the treatments of uveal melanoma.

**Methods** The eligible criteria for performing choroidectomy are the following: a basal dimension of ciliary-choroidal tumor up to 14 mm and a thickness for pre-equatorial choroidal tumor more than 6 mm. In our series 157 out of 326 patients presenting uveal melanoma were treated with conservative surgery: in 132 patients transscleral choroidecomy or cyclochoroidectomy was performed with or without adjunctive plaque brachytherapy. The aim of this study is to determine the safety and efficacy of the combined method (chorodectomy + plaque brachytherapy) compared to simple surgical resection. In our case series we selected 60 patients with similar uveal melanoma dimension (height 8 mm, basal dimension 10 mm); in 30 we performed choroidectomy and in other 30 we administrated adjunctive Ru 106 or Co 60 brachytherapy. The minimum follow-up was 24 months.

**Results** We analyzed surgery-related complications, local tumor recurrence and metastases. The surgery complications were similar; local recurrences in resection borders and in the central area were considerably lower in patients treated with combined thepapy. The incidence of liver methastasis was similar in both groups.

**Conclusion** As a conclusion we can state that based on our experience and according with the literature, the combined treatment (surgery + plaque brachytherapy) has an influence both on local recurrences and mortality.

■ **3142**

## Associated transpupillary thermotherapy and proton beam for posterior choroidal melanoma

*DESJARDINS l (1), DENDALE R (2), LUMBROSO l (1), LEVY C (1), DELACROIS S (3), NAURAYE C (3), PLANCHER C (4), ASSELAIN B (4)*
*(1) Ophthalmology, Paris*
*(2) Radiotherapy, Paris*
*(3) Physics, Orsay*
*(4) Biostatistics, Paris*

**Purpose** During protontherapy for posterior choroidal melanoma ,macula localized near tumor limit (<2.5mm) receive a great dose of radiation with an important risk of visual loss. In order to decrease macula toxicity with have reduced the safety margin for small posterior tumour, adding a limited treatment by transpupillary thermotherapy (TTT) on the posterior part of the tumour and adjacent retina .

**Methods** Only patient with posterior choroidal melanoma less or equal to 4 mm in thickness, with macula-tumour distance <2.5mm were selected. Three courses of TTT were delivered on posterior edge of the tumour and between tumour and macula with an infrared diode laser at 810nm and a beam diameter of 2 mm at 400 to 600mW. For proton irradiation, a 2.5 mm safety margin around tumour was taken, but it was reduced at 1.5mm near macula. The 90% isodose was always placed on that safety margin limit. The treatment delivered 60 Gy cobalt equivalent in four fractions on four consecutive days.

**Results** 12/1999 to 02/2003, 8 patients were included . Median age 56 yrs (45-88). Median tumour thickness : 3.1mm (2-4.1). Median tumour diameter : 11.4mm (8-14.4). .At follow up, all patients were still alive without local recurrence or metastatic disease. Pts 6 had a macular oedema, and pts 7 had undergone a surgery for cataract.With a mean follow up of 76 months the mean visual acuity is 20/33. 6 out of 8 patients have 20/25 vision .

**Conclusion** these results on a small prospective series of patients treated by an association of proton bream with localised safety margin reduction and limited TTT is encouraging

■ **3143**

## Comparison between Ruthenium-106 brachytherapy and sandwich therapy of Ruthenium-106 brachytherapy with Transpupillary Thermo Therapy (TTT) in 161 patients

*LY LV*
*Ophthalmology, Leiden*

**Purpose** To compare the effect of Ruthenium-106 brachytherapy with and without additive TTT on the occurrence of retinopathy, visual outcome, enucleation and other complications.

**Methods** Retrospective follow-up study of 80 choroidal melanoma patients treated with single brachytherapy (from 1985-1995) and 81 patients treated for choroidal melanoma with combination therapy (from 1995-1998) in the Leiden University Medical Centre, Leiden, The Netherlands. Tumour size was classified according to COMS criteria. Survival was estimated with the Kaplan-Meier analysis and Log Rank test. Retinopathy was analysed with the Chi-square test. Visual acuity was followed for four years.

**Results** The two groups were similar concerning age, initial visual acuity, prominence and location of the tumour. 28.8% of the tumours in the single treatment group and 22.2% in the combination group were classified as small, and 71.3% and 77.8% as medium (p=0.342), respectively.After 48 months of follow-up, mean visual acuity was not significantly different between the single treatment and the combination group(p=0.754). No significant difference (p=0.122) existed between the single treatment and the combination group in the occurrence of retinopathy (40.0% and 27.2%, respectively), or with regard to enucleation (10% and 4.9%, respectively (p=0.221)). The proportion of metastasis was 20.0% for the Ruthenium-106 group and 24.7% for the combination treatment group (p=0.475). The Log rank test showed no significant difference in survival between both groups (p=0.104).

**Conclusion** The outcome after combination therapy was not different from single treatment with regard to visual outcome, retinopathy, enucleation or survival rate.

■ **3144**

## Iodine 125 Episcleral Brachytherapy for Circumscribed Choroidal Hemangioma

*SAORNIL MA (1, 2), BIANCIOTTO C (1, 2, 3), BLANCO G (2), MUIñOS DíAZ Y (2), ALMARAZ A (4), DE FRUTOS JM (5, 6), GIRALDO A (7), LOPEZ-LARA F (5)*
*(1) Ocular Oncology Unit. Hospital Clínico Universitario, Valladolid*
*(2) Ocular Oncology Unit. Ophthalmic Pathology Registry "Miguel N Burnier". Instituto Universitario de Oftalmobiología Aplicada., Valladolid*
*(3) Fundación Carolina, Madrid*
*(4) Epidemiology and Preventive Medicine Department. Universidad de Valladolid, Valladolid*
*(5) Radiotherapy Department. Hospital Clínico Universitario, Valladolid*
*(6) Ocular Oncology Unit. Instituto Universitario de Oftalmobiología Aplicada., Valladolid*
*(7) Ocular Oncology Unit. Hospital Clínico Universitario., Valladolid*

**Purpose** To evaluate the efficacy of Iodine 125 episcleral brachytherapy in the treatment of circumscribed choroidal hemangiomas (CCH).

**Methods** A retrospective review of 8 consecutive patients with symptomatic CCH. Diagnosis was made by clinical ophthalmoscopic, angiographic and ultrasonographic features. Episcleral brachytherapy was performed with Ropes plaques charged with Iodine 125 seeds with a target apex dose of 40 to 50Gy. Best-corrected visual acuity (VA), presence of retinal detachment (RD), ultrasound measurements were registered at the time of the diagnosis and in the follow up.

**Results** All the patients were symptomatic with VA ranging from 0,7 to no light perception (NLP), and six presented with RD. Ultrasound mean largest base was 11,3 mm (7,8-14,3) and mean height 4,4mm (2,8-6,5). Follow up was 7 years in 2 cases and four years in 5 cases. Visual Acuity improved during the first year in 4 cases decreasing after the second year. All patients achieved complete resolution of retinal detachment without recurrences. All tumors decreased in height (5 cases < 2 mm in the last examination) and only 3 patients presented a localized and auto limited radiation retinopathy.

**Conclusion** Although new forms of treatment are showing to be effective in the early stages of circumscribed choroidal hemangioma, episcleral brachytherapy with I 125 play an important role in symptomatic cases with retinal detachment and large tumors.
Partially funded by grant "Jose Mª Aguilar Bartolomé"

■ **3145**

## Long-term survival in choroidal melanoma after 60Co brachytherapy

*ROMANOWSKA DIXON B, SZUSCIK I*
*Depatrment of Ophthalmology of Jagiellonian University, Krakow*

**Purpose** To evaluate the survival of patients treated 60Co brachytherapy for choroidal melanoma in Ophthalmological Department of Jagiellonian University.

**Methods** In this retrospective study 417 patients (188 women and 229 men) were treated from 1968 to 1996. The apex dose was 65- 200 Gy (av.120), exposure time 72-480 hours (av.227). The Kaplan-Meier method was used to estimate cumulative survival rates. Multivariate Cox proportional hazards modeling was used to identify prognostic clinical variables associated with the outcomes.

**Results** The 5-year survival based on all cases was 80%, 10-year 61% and 15-year survival 45%. The estimated survival time was 9 years.

**Conclusion** The age up to 65, mushroom shape, heavy pigmentation, anterior location and mixed type of the tumor were associated with the worse prognosis.

■ **3146**

## Dose, dose rate and secondary effects in episcleral brachytherapy

*DE FRUTO BARAJA JM (1, 2), SAORNIL MA (3, 4), HINCAPIE J (3, 4, 5), MUIñOS DíAZ Y (4), ALMARAZ A (6), LOPEZ-LARA F (7), GIRALDO A (3)*
*(1) Radiotherapy (Medical Physics), Hospital Clinico Universitario, Valladolid*
*(2) Ocular Oncology, Instituto Universitario de Oftalmobiología Aplicada, Universidad de Valladolid, Valladolid*
*(3) Ocular Oncology, Hospital Clinico Universitario, Valladolid*
*(4) Ocular Oncology and Ophthalmic Pathology Registry "Miguel N Burnier". Instituto Universitario de Oftalmobiología Aplicada. Universidad de Valladolid, Valladolid*
*(5) Fundación Carolina, Madrid*
*(6) Epidemiology and Preventive Medicine, Universidad de Valladolid, Valladolid*
*(7) Radiotherapy and Oncology, Hospital Clinico Universitario, Valladolid*

**Purpose** To correlate the dose and the dose rate in the organs of risk with the secondary effects in these locations and with the overall secondary effects.

**Methods** Prospective study of 76 eyes of patients diagnosed of choroidal melanoma and treated with Iodine 125 epiescleral brachytherapy bewteen 1997 and 2004 were analyzed. Brachytherapy was performed with Ropes applicators. The dose distribution was calculated with the Bebig planning system. Secondary effects and the dose and the dose rate in the organs of risk for ach case were registered in a Access data base. Statistical treatment of the data was performed with SPSS and statistical descriptive and t of Student and coefficient of correlation of Pearson were applied.

**Results** The 50 percent of the patients presented some type of complication, being the most numerous radiation retinopathy(26 % ) and cataract (21 % ). Statistical significance has been found between: the cataract and the dose rate in crystalline, macular edema and the dose in fovea, between rubeosis and the dose to 6 mm of the applicator and in esclera and the dose rate in esclera. The risk of developping any secondary effects correlates with the dose in lens, the center of the eye, esclera, and to 6 mm of the center of the applicator and the dose rate in apex, center of the eye, and dose to 6 mm of the applicator.

**Conclusion** There is correlation with statistical significance between the appearance of secondary effects and the dose and dose rate in reference points as center of the eye and to 6 mm of the plaque. Partially funded by grant "José Mª Aguilar Bartolomé" and FIS 01/1664 (Ministerio de Sanidad y Consumo, España)

■ **3147**

## Survival of largest uveal melanomas after primary enucleation

*GAMBRELLE J, KODJIKIAN L, GRANGE JD*
*Croix-Rousse Hospital, Lyon*

**Purpose** Our main goal was to evaluate survival of primarily enucleated melanomas since the advent of proton beam therapy in France in 1991.

**Methods** Posterior uveal melanomas treated by primary enucleation in our department between 1991 and 2002 have been included in this retrospective study. 5-year melanoma-specific survival was calculated according to the Kaplan-Meier method. The multivariate prognostic analysis was performed using the Cox proportional hazards model.

**Results** Forty patients, representing 8 % of patients diagnosed and followed in our department during the period 1991-2002, were included in our study. No patient had been lost of follow-up. The 5-year melanoma-specific survival rate was 31.45 % (SE: 7.8) after primary enucleation. Significant prognostic factors for death in multivariate analysis were occurrence of metastasis ($p<0.0001$), tumour thickness > 12 mm (p: 0.019), iris root involvement (p: 0.023) and presence of epithelioid cells (p: 0.017).

**Conclusion** Our results highlight the pejorative prognosis for the largest tumours for which primary enucleation remains the reference treatment. The majority of recent published reports have analysed melanoma-specific survival only in populations that have been treated by conservative radiotherapies, without mentioning the proportion of melanomas which were primarily enucleated. However, not taking into account the primarily enucleated population biases the comparison with studies that pre-date the advent of eye-preserving therapies. Moreover, it leads to an implicit overestimation of the melanoma-specific survival of the entire population.Finally, our low rate of survival demonstrates that the enhancement of this cancer detecting strategies remains a key goal for Health programs.

■ 3151

## Towards a standardised technique for meibometry

*TIFFANY JM (1), LAVRIC A (2), YOKOI N (3)*
*(1) Nuffield Laboratory of Ophthalmology, Oxford University, Oxford*
*(2) Ophthalmology, University Medical Centre, Ljubljana*
*(3) Ophthalmology, Prefectural University of Medicine, Kyoto*

**Purpose** To simplify and standardise equipment and procedures for the technique of meibometry (estimation of the amount of lipid on the eyelid margins by blotting with plastic tape and measuring the change in optical density).

**Methods** Parallel blots (L eye/R eye) were taken by the old method (10 sec application at 15g pressure) or by a 3-sec touch at zero pressure. Two zero-pressure methods were tried: fixing the tape in the prism mounting of a Goldmann tonometer, or attached by Blu-Tack adhesive to the end of a thin bamboo skewer, held upright by Blu-Tack on the Goldmann mounting plate. All blots were read in the Courage & Khazaka Meibometer® instrument, but also scanned with mirror backing by a flat-bed or an IRIS-Pen hand-scanner and the grey-scale image quantified by computer densitometry.

**Results** No significant difference was found between the two types of zero-pressure mounting. Less oil was picked up by the zero-pressure method than the 10-second method, but the amount was adequate in both cases; subjects preferred the zero-pressure touch, finding it less invasive as the loop of tape was easier to position on the lid. Use of the Meibometer gave immediate results but scanning, especially with the IRIS Pen, is more suitable for field studies as images can be stored before reading with no post-sampling degradation of tapes. Images from a flat-bed scanner were equally acceptable.

**Conclusion** Apart from the meibometry tape (obtainable only from Courage & Khazaka), no special equipment is needed as scanners and PCs are readily available, as is suitable free densitometry software, e.g. from NIH/Scion. Simpler mounting of the tape loop, shorter blotting time and more precise placement, and rapid capture of a grey-scale image of the blot all contribute to a more standard technique.

■ 3152

## A Comparison of Systane® Lubricant Eye Drops versus Refresh Tears Lubricant Eye Drops when used as Supportive Therapy with Cyclosporine (0.05%) Ophthalmic Emulsion

*PRZYDRYGA J (1), SALL K (2), COHEN S (3), CHRISTENSEN M (1)*
*(1) Alcon Labs, Fort Worth, TX*
*(2) Sall Eyecare, Bellflower, CA*
*(3) Private Practice, Scottsdale, AZ*

**Purpose** To evaluate the efficacy and compatibility of two marketed tears in relieving dry eye signs/symptoms when used as supportive therapy to a cyclosporine based ophthalmic emulsion.

**Methods** 60 patients were evaluable by intent-to-treat analysis for this investigator masked, parallel study of 6-months duration. Enrollment included corneal staining of > 3 (NEI grid), Schirmer w/o anesthesia of < 7mm and subjects had to answer that they needed artificial tears at least "some of the time". Subjects were randomized to one of 3 treatment groups. Treatment (Tx)1: Cyclosporine (0.05%) BID w/Systane® (PEG 400/propylene glycol w/HP-Guar as a gelling agent) used a minimum of 1/day as supportive therapy. Tx2: Cyclosporine BID w/Refresh Tears® (carboxymethylcellulose) used a minimum of 1/day as supportive therapy. Tx3: Systane used alone QID. Signs/symptoms were measured at Days -7, 0, 7, 14, 28, 42, 120 and 180.

**Results** A statistical difference was seen in favor of Tx1 (Cyclosporine+Systane) vs Tx2 (Cyclosporine+Refresh) for greater reduction in corneal staining (p=0.0048) and a trend (p=0.0725) for increased TFBUT at 6 months. There were no differences in Schirmer scores at six months: Tx1=1.41, Tx2=2.15, Tx3=1.42 mm. Significant differences were seen in favor of Tx1 vs Tx2 for less frequent ocular burning (p=0.0210), stinging (p=0.0314), grittiness (p=0.0128) & dryness (p=0.0132). Tx3 (Systane alone) was better than Tx2 (Cyclosporine+Refresh) for less frequent ocular burning (p=0.0288), dryness (p=0.0480) & scratchiness (p=0.0294).

**Conclusion** The choice of tears used as supportive therapy with a Cyclosporine Ophthalmic Emulsion has significant indications for outcome measures. There were significant clinical advantages with Tx1 vs Tx2. There were no clinical or statistical differences seen between Tx1 vs Tx3 (Systane used alone). Both supportive therapies were compatible with the Cyclosporine Ophthalmic Emulsion. This study was sponsored and conducted by Alcon Research Ltd, Fort Worth, TX USA.

■ 3153

## Efficacy of an HP-Guar based lubricant eye drop in the treatment of dry eye disease

*MESSMER EM, GASS CA*
*Department of Ophthalmology, Ludwig-Maximilians-University, Munich*

**Purpose** To evaluate the efficacy of a new lubricant eye drop containing polyethylene glycol 400 and propylene glycol demulcents with HP-guar as a gelling agent (Systane) for reducing dry eye symptoms

**Methods** 20 patients with dry eye symptoms and the need to use artificial tears were enrolled in one center for an 6 week, open-label clinical study. 83% of the patients were female with a mean age of 47 years. Follow-up visits were performed after 1 and 6 weeks. The frequency and severity of symptoms was assessed using an analog scale from 0 (none) to 5 (continuously) for frequency and from 0 (none) to 3 (severe) for severity. The ocular comfort associated with the assigned lubricant was measured using a 5-point Likert-format scale. Objective signs of dry eye were assessed using lissamine and fluorescein staining of the conjunctiva and cornea. Statistics were performed using Wilcoxon Tests.

**Results** The study medication was comfortable and refreshing in 100 and 94% of patients, respectively at week 1 and 6. It made patients forget their dry eye symptoms in 83% after 1 week and 100% after 6 weeks. The frequency of overall symptoms decreased significantly after 1 and 6 weeks. The severity of foreign body feeling, the severity of dryness, and the severity of itching were significantly reduced in comparison to day 0. Lissamine staining of the conjunctiva decreased significantly after 6 weeks (p=0.002). Fluorescein staining of the inferior cornea was significantly reduced 1 and 6 weeks following treatment (p</=0.004). Tear film break up time significantly increased after 6 weeks (p=0.016).

**Conclusion** Systane showed an overall good tolerability. Moreover, it was able to decrease objective signs and subjective symptoms of dry eye disease significantly.

■ 3154

## Corneal sensitivity and nerve morphology in primary Sjögren´s syndrome

*TUISKU IS (1), KONTTINEN YT (2, 3, 4), TERVO TM (1)*
*(1) Helsinki University Eye Hospital, Helsinki*
*(2) Helsinki University, Department of Medicine, Helsinki*
*(3) Orton Orthopaedic Hospital of the Invalid Foundation, Helsinki*
*(4) COXA Hospital for Joint Replacement, Tampere*

**Purpose** To correlate corneal sensitivity threshold values in primary Sjögren's syndrome (pSS) to subjective symptoms, objective clinical signs and subbasal nerve density.

**Methods** Twenty eyes of 20 pSS patients (mean age 54.5 + 7.0 years) were studied. Subjective dry eye questionnairy (OSDI) was used and clinical objective signs of dry eye were assessed. The central corneal mechanical sensitivity was assessed with modified Belmonte Non-Contact Esthesiometer. Corneal nerve morphology and density was assessed using confocal microscopy (ConfoScan 3).

**Results** The mean corneal sensitivity threshold value was lower in pSS (55.1 + 39.2 ml/min) than in the controls (91.4 + 23.4 ml/min; p = 0.030) indicating hypersensitivity. Ocular surface disease index (OSDI) score for dry eye symptoms was significantly higher in pSS patients (41.6 + 24.0 %) compared to normal controls (8.0 + 6.6;p = 0.001). Corneal nerve densities were similar (5.8 + 2.2 in pSS vs. 5.8 + 3.0 in controls, p = 0.988). Interestingly, no correlation was found between corneal sensitivity and nerve density, but corneal nerve morphology was altered in pSS. Nerve growth cone- like structures were seen in 20 % and Langerhans cell –like structures in 7/20 (35 %) patients.

**Conclusion** Corneal hypersensitivity in primary Sjögren's syndrome did not correlate with corneal nerve density. Instead, alterations seen in the subbasal nerve plexus may contribute to hyperalgesia and clarify the discrepancy clinically often seen between objective signs and subjective symptoms in these patients.

■ 3155 / 231

## Presence of IgE Antibody and Kinin Components in Atopic and Non-Atopic Contact Lens Wearing Patients

*MANN AM, TIGHE BJ*
*Biomaterials Research Unit, Birmingham*

**Purpose** The anterior ocular surface is a common target in the allergic response, however very little detail is known about the mechanisms involved or the added complications that ensue during, or as a consequence, of contact lens wear. The present work incorporates a variety of studies in which IgE and kinin activity was determined in contact lens wear and also provides an insight into their individual detection profiles and synergistic interactions. Additionally as a second objective, complementary studies are introduced which analyse the individual tear protein profile of patients using the very sensitive and reproducible results acquired on the 2100 Agilent Bioanalyser.

**Methods** The extracted lens deposits and tear 'fingerprints' were analysed by immunodiffusion assay and protein sizing lab-on-a-chip technology respectively. A variety of contact lenses over a range of modalities and materials were tested in both non-atopic subjects and those with existing allergies.

**Results** There was a progressive and significance increase in the percentage of subjects presenting with kinin and IgE moieties in their lens deposits between months one and twelve months. IgA levels were distinctly different between the control, normal subjects and those identified with allergies; the levels of tear IgA for the normal subjects were in the region of 425[FU] whereas the atopic subjects presented levels of approximately 125[FU]. Changes in IgA levels over time in contact lens wearers was determined to be patient and symptom dependent.

**Conclusion** The assessment and analysis of immunological changes in the tear film and during contact lens wear is important in discerning ocular complications.

■ 3156 / 232

## Expression Of Focal Adhesion Protein PINCH In Alkali Injured Corneas And Role Of PMNs

*BOURGHARDT PEEBO B, GAN L, FAGERHOLM PPP*
*Dept. of Ophthalmology, University Hospital, Linköping*

**Purpose** Particularly interesting new cystein-histidin-rich protein (PINCH) functions as an adapter protein for signal transduction in the integrin and growth factor pathway. The purpose is to study the expression of PINCH in normal versus alkali wounded corneas and the influence of PMNs.

**Methods** The right eye of 14 New Zealand White rabbits were injured with a central penetrating 5 mm in diameter alkali injury in general anaestesia. Seven of the rabbits were injected intravenously with fucoidin every two hours for 36 hours. All rabbits were sacrificed after 36 hours and the corneas were excised with a scleral rim and fixed in 4% formaldehyde and embedded in paraffine. 4 µm thick sections were then immunohistochemichally stained using a polyclonal PINCH antibody.

**Results** PINCH was not expressed in the unwounded normal cornea or in the vascular endothelium of the limbal vessels. In the injured cornea the cells repopulating the wound after 36 hours, epithelium, stroma and endothelium all expressed PINCH in the frontline. In the limbal area the expression of PINCH was extensive in stromal, vascular endothelial and inflammatory cells. In the corneas from fucoidin injected rabbits, the expression of PINCH was positive in the repopulating cells although weaker. In the limbal area the expression of PINCH was strongly reduced but still somewhat more discernible in the stromal cells compared to unwounded corneas.

**Conclusion** PINCH is a marker for several types of cell activities and is, like in tumor growth, expressed during wound healing. PINCH is also expressed in the limbal vascular endothelium when there is stimulation for neovascularization. The absence of PMNs prevents the expression of PINCH in the vascular endothelium.

## ▪ 3161
## Mouse Vision Phenotyping

*SCHAEFFEL F*
*University Eye Hospital, Section Neurobiology of the Eye, Tuebingen*

**Purpose** The mouse may be an important model to study the genetic and environmental influences on refractive development, because its genome has been extensively studied, many genetic knock-out models are available and because it can be easily bred. However, its eyes are small (about 3 mm axial length), their optical quality is not very good and spatial acuity is not very high. To study myopia development and visual function, we have established a battery of new techniques.

**Methods** Automated infrared photorefraction was adapted for mice. Optical low coherence interferometry (OLCI) was adapted to measure ocular dimensions with a with a standard deviation of only about 15 µm. Visual acuity was determined in a "whole body optomotor paradigm" in unrestrained mice by a self-programmed video tracking technique, in which both angular speed and body orientation of mice were tracked. Retinal function was determined by a self-programmed automated pupillography.

**Results** Only about 2-3 dopters of myopia could be induced in eyes of young mice that were covered for two week with a translucent frosted diffuser (n=50, age 40 days) but OCLI confirmed that the eyes had elongated by a few micrometers. Grating acuity was about 0.4 cyc/deg in the whole body optomotor paradigm. It declined with decreasing ambient illuminance, studies in mutants lacking rods or cones showed that the rods are providing the best spatial acuity. Grating acuity was affected by defocus imposed by spectacle lenses only if the lens power exeeded +/-10 D, indicating a huge depth of field. Pupillography showed that one atropine eye drop (1%) reduces pupil responses for 2 weeks.

**Conclusion** Using these techniques, it is possible to study the effects of defined knockout of genes and of pharmacological intervention on both myopia development and visual function.

## ▪ 3162
## Corneal curvature in the mouse

*VOTRUBA M (1, 2)*
*(1) School of Optometry & Vision Sciences, Cardiff University, Cardiff*
*(2) The University Hospital Wales, Cardiff*

**Purpose** To describe the methods available for the assessment of corneal curvature in the mouse eye and how this contributes to phenotyping in studies of mouse models of genetic eye disease, with examples of concrete mouse mutants.

**Methods** A variety of modifications can be made to keratometry methods used in humans, including hand held placido discs with photography, infra red photokeratometry and video keratoscopy.

**Results** Measuring corneal curvature in the mouse is a challenging technique. It is needed to further our understanding of eye growth and emmetropisation, as well as for the comprehensive phenotyping of mouse models of corneal ectasia. These models are still relatively few, but include the keratoconus/ vsx1 knockout mouse, the cornea plana/ keratocan knockout and lumican mice, findings in which are described.

**Conclusion** Corneal curvature forms an useful adjunct in the evaluation of the eye of any animal model with purported effects on eye development or function. It will be essential as models of corneal ectasias, such as keratoconus, keratoglobus or cornea plana are generated, and useful in models of corneal dystrophies and studies of eye growth and development.

## ▪ 3163
## OLCI: a new tool to screen for mouse mutants, which are affected in eye growth

*PUK O, DALKE C, GRAW J*
*GSF - National Research Center for Environment and Health, Institute of Developmental Genetics, Neuherberg*

**Purpose** Aim of the study is the determination of natural variations in ocular dimensions and the identification of new mouse mutants characterized by altered eye size.

**Methods** The growth of the axis length in general and the lens in particular of animals from different inbred strains were measured with optical low coherence interferometry (OLCI) in the period between 4 and 15 weeks. Offspring from ENU-treated animals were analyzed at the age of 11 weeks for altered ocular dimensions.

**Results** The OLCI is an effective tool to detect and establish new mouse mutant lines which are slightly affected in the eye size. In order to recognize these types of mutants, it is necessary to identify the natural variation of ocular dimensions in wild type strains. Therefore we determined the length of the optical axis in 4 strains (C3HeB/FeJ, C57BL/6J, BALB/cByJ, 129S6/SvPas) between 4 and 15 weeks. C3HeB/FeJ was observed to have significant longer axes (3.61±0.03 mm, at 15 weeks) and significant thicker lenses (2.26±0.02 mm) than animals of the other lines (3.55±0.03 mm; 2.16±0.03 mm), which showed no significant differences in these values. Using the OLCI, 22 offspring of an ENU screen have now been analyzed in the exponential growth phase of the eye (11 weeks). We detected 5 animals showing significant alterations in ocular dimensions. These variations are tested whether they are eye size mutations.

**Conclusion** The OLCI data of axial- and lens growth in animals of the inbred strains C3HeB/FeJ, C57BL/6J, BALB/cByJ, and 129S6/SvPas revealed only small size variations in animals of the same strain and at the same age. With the determined baseline data it is now possible to screen for variations and to establish new mutants which are only slightly affected in their eye size.

## ▪ 3164
## Measurement of intraocular pressure in mice

*REITSAMER HAR (1, 2)*
*(1) Ophthalmology and Optometry, Salzburg*
*(2) Physiology, Vienna*

**Purpose** The Tonopen® is a commercially available hand help applanation tonometer for human use. However, Moore and co-workers proved its validity in a rat model of ocular hypertension (Invest Ophthalmol Vis Sci. 1993;34(2):363-9) providing the impetus for this study to standardize a method of non-invasive measurement of intraocular pressure (IOP) in mice.

**Methods** Two studies were performed: In the cannulated-eye study IOP was measured simultaneously with a Tonopen® and by direct cannulation of the vitreous compartment while pressure was manipulated in steps between 10 and 45 mmHg by a saline reservoir via a second vitreal cannula (five mice, one rat). In a second study Tonopen and servo-null measurements were performed in independent groups (48 mice) to verify Tonopen measurements in non-cannulated-eyes. In this study Topical brimonidine (0.15%) was used to decrease IOP.

**Results** In the rat, there was a similar relationship between Tonopen readings and direct measurements via cannulation of the eye as previously reported. Although readings from mice eyes were higher in variability than those obtained from the rat, the measurements were reproducible and the correlation between the invasive and the non-invasive methods was good ($r = 0.97$). The IOP lowering effect of brimonidine was detected with the Tonopen as well as with servo-null measurements ($p < 0.001$) and the results with both techniques were similar.

**Conclusion** The Tonopen can be used for rapid and reproducible measurements of IOP in mice. The method can provide a useful means for IOP measurement in mouse models of induced ocular hypertension, in knock-out and transgenic mice, or in pharmacological studies.

■ **3165**

## Screening mice for vision

*DALKE C (1), FAVOR J (2), PUK O (1), GRAW J (1)*
*(1) Institute of Developmental Genetics, Neuherberg*
*(2) Institute of Human Genetics, Neuherberg*

**Purpose** To screen mice for vision capability efficient and reliable methods are needed.

**Methods** Slit lamp biomicroscopy is used to recognize morphological alterations in the anterior segment of the eye; it is extended by fundoscopy for the investigation of the posterior part of the eye. The eye size of mice is measured by low coherence interferometry, with the AC-Master (Zeiss). For screening purposes, a high throughput electroretinography (ERG) was established as a functional test for retinal abnormalities. Histological analysis follows standard procedures. To measure the intra ocular pressure of the eye an applanation tonometer was tested.

**Results** Generally, the equipment for eye examinations is adapted to humans. To use this equipment for mice, it needs to be tested for suitability. Not just the much smaller eyes of mice, but also animal handling during the examination must be considered. For a large-scale, standardized vision analysis of mice from various sources (e.g. transgenes, knockout mice, ENU mutants) we choose different non-invasive methods in a hierarchical test protocol. In a primary screen the eyes are examined morphologically with the slit lamp and the fundoscope, which are quick and easy to use instruments for experienced examiners. Additionally, the eye size (axial length) is determined to complete the analysis. If alterations were found in the primary screen, ERG as a functional test of the retina and histological analysis of the eye will be performed as a secondary screen.An applanation tonometer was tested, but was not considered as usable for mice.

**Conclusion** A comprehensive vision analysis of mice can be performed in a primary screen with basic methods and confirmed by secondary screens that are more time-consuming methods.

## ▪ 3211
## Study design in clinical ophthalmology

*SAW SM*
*Community, Occupational and Family Medicine, National University of Singapore, Singapore*

**Purpose** To outline the designs of cross-sectional, case-control and cohort studies.

**Methods** To describe the general purposes, methodology and design of different epidemiologic studies, including cross-sectional, case-control and cohort studies.

**Results** Examples of cross-sectional, case-control and cohort studies from the ophthalmic literature will be highlighted.

**Conclusion** Important study designs used to address exposure-disease associations will be explored.

## ▪ 3212
## Understanding basic statistics in ophthalmology

*SAW SM*
*Community, Occupational and Family Medicine, National University of Singapore, Singapore*

**Purpose** To describe and outline the statistical approaches for the analysis of clinical, epidemiologic and laboratory studies.

**Methods** This workshop will outline the types of variables (categorical, ordinal or continuous) used in statistical analysis. Hypothesis testing and interval estimation will be conducted to compare 2 proportions (chi-square test and Fisher's exact test), 2 means (t test), and 2 medians (Wilcoxon ranksum test). Both parametric (t test) and non-parametric tests (Wilcoxon ranksum test) will explained.

**Results** Examples of statistical analysis from other relevant eye studies will be cited.

**Conclusion** Both descriptive and bivariate analyses will be summarized using relevant examples from eye research.

## ▪ 3213
## The design and conduct of randomized controlled trials

*SAW SM*
*Department of Community, Occupational and Family Medicine, National University of Singapore, Singapore*

**Purpose** To summarize the design and unique features of a randomized clinical trial

**Methods** The general study design of a randomized clinical trial will be outlined. The importance and advantages of randomization and masking will be discussed. Follow-up, biases, and analysis of randomized clinical trials will be described.

**Results** Examples of clinical trials in ophthalmology will be cited.

**Conclusion** The unique features, importance and usefulness of randomized clinical trials will be outlined.

■ 3221

## Optic disc hemorrhages and progression to glaucoma in the European Glaucoma Prevention Study (EGPS)

*STALMANS I (1), ZEYEN T (1), MIGLIOR S (2), PFEIFFER N (3), CUNHA-VAZ J (4), ADAMSONS I (5), EGPS STUDY GROUP*
*(1) Ophthalmology, Katholic University Leuven, Leuven*
*(2) Ophthalmology, Universita Milano Biccocca, Monza*
*(3) Ophthalmology, Meinz University, Meinz*
*(4) Ophthalmology, Azinhaga Santa Coimba, Coimbra*
*(5) Ophthalmology, Lafayette Hill, Lafayette Hill*

**Purpose** To assess the sensitivity of fundoscopy and evaluation of optic disc stereophotographs to detect optic disc hemorrhages (ODHs). To determine the predictive value of ODHs for the progression to glaucoma.

**Methods** The European Glaucoma Prevention Study was designed to evaluate whether reducing the intra-ocular pressure in patients with ocular hypertension (OHT) might reduce the risk of progression to glaucoma. All eyes included in the EGPS study were monitored for optic disc hemorrhages (ODHs) every 6 months for 5 years both by fundoscopy and evaluation of optic disc stereo-photographs. Chi-square test was used to compare the rates of progression.

**Results** 1342 eyes of 750 participants (drop-outs excluded) were analyzed. Out of 30 disc hemorrhages in total, 7 (23,3%) were discovered by fundoscopy and 29 (96,7%) were detected on stereophotographs. Eight patients (26,7%) with a disc hemorrhage progressed to glaucoma, whereas 98 patients (7,5%) of patients without disc hemorrhages reached this endpoint ($p<0.001$). The ODHs that were detected by fundoscopy lead to an endpoint in 71,4% of patients and the ones that were discovered on stereophotos in 24,1% of patients.

**Conclusion** These results indicate that reading stereophotos may be more sensitive than fundoscopic examination to detect ODHs. Disc hemorrhages are associated with an increased risk of progression to glaucoma, particularly when detected during fundoscopy.

■ 3222

## Early Changes in Sub-retinal Layers of the Macular Region and the Optic Nerve Head in Ocular Hypertensive Non–Human Primates

*DOELEMEYER A (1), POLSKA EA (2), SCHMETTERER L (2, 3), DREXLER W (3)*
*(1) Novartis Institutes for BioMedical Research, Basel*
*(2) Department of Clinical Pharmacology, Medical University of Vienna, Vienna*
*(3) Center for Biomedical Engineering and Physics, Medical University of Vienna, Vienna*

**Purpose** To assess early morphological changes in the retina in eyes of cynomolgous monkeys with unilaterally induced ocular hypertension (OHT).

**Methods** 6 cynomolgous monkeys were included. OHT was induced unilaterally. 3 baselines were performed and study days were scheduled every 2-3 weeks over 12 month. Measurements included intraocular pressure (IOP), ultrahigh–resolution optical coherence tomography (uhr–OCT), scanning laser polarimetry (GDx) and confocal scanning laser tomography (HRT). Cross-sectinal images of the macula and the optic nerve head (ONH) were performed with the uhr-OCT system. The GDx system with individual variable cornea compensation was used to construct nerve fiber layer (NFL) thickness maps.The topography of the ONH was measured using the HRT.

**Results** The 6 monkeys showed a diverse development of OHT. The ONH morphology was altered in all 6 monkeys. Stable elevation of IOP by >20 mmHg caused an almost complete loss of NFL in 6-8 weeks after induction of OHT (evidenced by GDx). The decrease in NFL thickness could be confirmed with the uhr-OCT, also showing a marked reduction in ganglion cell layer thickness in the macula area. In 1 monkey no loss of NFL could be evidenced despite a stable elevation of IOP. In the 3 remaining monkeys only a sporadic and/or mild elevation of IOP could be observed, leading to morphological changes in the ONH of much lesser degree.

**Conclusion** Morphological changes can be observed early after induction of OHT. The data from the different measurement modalities correlate well with each other. uhr-OCT allows detection of sub-retinal layer thinning already at early stages of disease progression in the monkey model of glaucoma.

■ 3223

## Electroretinographic and retinal lipid modifications in a rat model of glaucoma

*BRON AM (1), ACAR N (2), ARNAVIELLE S (1), GREGOIRE S (2), JOFFRE C (2), BRETILLON L (2), GARCHER CREUZOT CP (1)*
*(1) Ophthalmology, University Hospital, Dijon*
*(2) Eye and Nutrition Research Group, Dijon*

**Purpose** To evaluate the functional changes and the lipid profile in the retina of a chronic rat model of glaucoma.

**Methods** Six-week-old Brown Norway rats were used. Elevation of IOP was obtained by means of an injection of silicone in the anterior chamber of one eye. The other eye served as control. IOP was then recorded during 30 weeks with a Tonopen®. The functional evaluation of the retina was done by electroretinography (ERG) before and 12 and 30 weeks after the injection of silicone. Animals were sacrificed and the retinas were isolated. After the extraction of retinal lipids, the fatty acid composition was determined by gas chromatography (GC) with flam ionization detection (FID).

**Results** Elevation of IOP was sustained during the whole experiment. The ERG results did not show any significant difference in b-wave latency. However, the b-wave amplitude was reduced at 12 weeks (-37% vs -19%) and at 30 weeks (-81% vs -75%) in the treated and control eyes respectively. The retinal content of fatty acids did not vary between injected and control eyes. However, the DHA levels were 23% of total fatty acids whereas they are about 30-33% in normal rat retinas.

**Conclusion** These results show that the silicone injection in anterior chamber is a reliable model to induce a chronic model of elevation of IOP. The impairment of ERG as well as the partial depletion of DHA in control eyes suggests a possible controlateral effect of the elevation of IOP.

■ 3224 / 337

## Central corneal pachymetry in children with congenital glaucoma after trabeculotomy

*OBERACHER-VELTEN I, LORENZ B*
*Dept. of Paediatric Ophthalmology, University Eye Hospital, Regensburg*

**Purpose** It was the aim of the present study to investigate the value of corneal pachymetry in children with congenital glaucoma after trabeculotomy.

**Methods** Twelve eyes of nine children with congenital glaucoma had follow-up examinations with corneal ultrasound pachymetry, applanation tonometry, and slit-lamp biomicroscopy before and for at least three months after trabeculotomy. Corneal pachymetry, slit-lamp biomicroscopy, and whenever possible applanation tonometry were performed without general anaesthesia.

**Results** Seven of twelve eyes had biomicroscopically clear corneas without visible corneal edema before trabeculotomy. Mean central corneal thickness was 680 ± 57 µm before trabeculotomy and decreased to 597 ±45 µm after trabeculotomy. Mean intraocular pressure (IOP) was 22 ± 5 mmHg before and decreased to 12 ± 4 mmHg after trabeculotomy. The decrease of central corneal thickness was significantly correlated with the decrease in IOP. Even those eyes with biomicroscopically clear corneas before trabeculotomy showed a decrease in central corneal thickness after trabeculotomy.

**Conclusion** Central corneal pachymetry seems to be a useful additional information in children with congenital glaucoma after trabeculotomy. As corneal ultrasound pachymetry easily could be performed without general anaesthesia in all children and often can be done more easily than applanation tonometry, it can be taken as additional follow-up examination to control the IOP regulation in children with congenital glaucoma.

■ 3225

## Correlation between Ocular Pulse Amplitude and Visual Field Defects

*VULSTEKE C (1), ZEYEN T (2)*
*(1) Ophthalmology, Middelheim Hospital, Antwerp*
*(2) Ophthalmology, University Hospital, Leuven*

**Purpose** To investigate the correlation between the Ocular Pulse Amplitude (OPA) measured by Pascal Dynamic Contour Tonometer (DCT) and visual field defects in patients with Primary Open-Angle Glaucoma (POAG), Ocular Hypertension (OHT), and in Glaucoma Suspects (GS).

**Methods** Hundred eyes of 52 patients were examined. OPA was measured with DCT by one investigator masked to the visual field data. Only measurements of quality q1 and q2 were included. Visual fields were performed by Humphrey (n=52) or Octopus (n=32), and were analysed with Peridata software. Mean defect (MD), pattern standard deviation (PSD; Sqrt LV), and regression analysis of MD and PSD (Sqrt LV ) (Trend indices) were correlated to OPA for each eye. Spearman correlation coefficients were used to assess the correlation.

**Results** Fifty-three eyes had POAG, 14 had OHT, and 17 were GS. Regression analysis of MD and PSD (Sqrt LV) was available in 62 (73,8%) and 57 (67,9%) eyes respectively. The mean follow-up was 46 (6-96) months. There was a statistically significant correlation, both for Humphrey (n=52) and Octopus (n=32), between OPA and MD (respectively rs = 0,681 and rs = 0,670, p<0,001), and between OPA and PSD (Sqrt LV) (respectively rs = -0,463 and rs = -0,629, p<0,001). There was a weak, but statistically significant correlation between OPA and the regression analysis of MD (rs =0,250, p=0,05). The correlation between OPA and the regression analysis of PSD (Sqrt LV) was rs =-0,184, p=0,17.

**Conclusion** A small ocular pulse amplitude might be a risk factor for the development and progression of glaucomatous visual field defects.

■ 3226 / 338

## Retrobulbar hemodynamics in asymmetric glaucoma

*REMKY A, PLANGE N, KALIP M, AREND O*
*Universitäts-Augenklinik, Technical University Aachen, Aachen*

**Purpose** Previous studies investigated blood flow velocities of retrobulbar vessels in patients with glaucoma. Most of the studies confirmed reduced end-diastolic velocities associated with elevated resistive indices in the central retinal artery and the posterior ciliary arteries. This study was performed to correlate the degree of glaucomatous damage with retrobulbar hemodynamics in patients with normal tension glaucoma (NTG) and primary open-angle glaucoma (POAG) by comparing in the same patient the eye with advanced visual field loss with that with no ore mild field loss.

**Methods** 25 POAG patients and 15 NTG patients exhibiting asymmetric visual field loss (difference of mean deviation between the eyes >6dB) were included in this prospective 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 color Doppler imaging in both eyes.

**Results** Mean deviation of eyes with more severe glaucomatous visual field loss was –17.1dB versus –5.6dB (p<0.0001). Intraocular pressure and perfusion pressure were not significantly different between eyes. The PSV of the OA (30.5 ±8cm/s versus 32.9 ±9cm/s, p=0.014) and the EDV of the CRA (2.2 ±0.4cm/s versus 2.5 ±0.5cm/s, p<0.005) were significantly decreased in eyes with more severe glaucomatous field loss. The diagnosis (either POAG or NTG) had no influence on hemodynamic data.

**Conclusion** Patients with POAG or NTG and asymmetric glaucomatous visual field loss exhibit asymmetric blood flow velocities of the OA and CRA. The posterior ciliary arteries did not show any significant differences between the more and less affected eye.

■ 3227

## Measurement of cytokine secretion by multiplex bead analysis in tears of glaucoma patients with preserved topical treatment

*GARCHER CREUZOT CP (1), MALVITTE L (1), LAFONTAINE PO (1), MONTANGE T (2), LIZZARD G (2), BRON AM (1)*
*(1) Department of Ophthalmology, Dijon*
*(2) Unité INSERM U498, Dijon*

**Purpose** To investigate the ocular surface inflammatory response to long term instillation of local preserved treatments in glaucoma patients using measurement of cytokines in tears. The multiplexed bead analysis allows the measurement of pro-inflammatory, Th1-type, Th2-type and chemokine production on a single tear sample and requires a small volume.

**Methods** Tear samples were collected from 14 glaucoma patients compared to 7 healthy normal volunteers with no history of ocular surface disease. Two µl of tears were withdrawn with a capillary tube, diluted in 48µL of PBS and stored at -80°. The simultaneous measurement of 17 cytokines in one tear sample was performed by multiplexed bead analysis using microspheres as the solid support for immunoassays (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, G-CSF, GM-CSF, INFγ, MCP-1, MIP-1β, TNFα).

**Results** The secretion of pro-inflammatory cytokines (IL-1β, IL-6, IL-12, TNFα) was increased (p<0.05) in treated patients. The Th-1type cytokines were more expressed in tears of treated patients than the Th2- type (p<0.05) whereas this difference was not observed in controls. The chemokine expression (IL-8 and MCP-1) was also increased in the treated group (p<0.05).

**Conclusion** The present study demonstrates that pro-inflammatory cytokine secretion by conjunctival cells is increased in response to glaucoma topical treatments. The multiplexed analysis is a promising method to measure multiple cytokine and chemokine production in tears.

## ▪ 3231
## Physiopathology of macular oedema

*AGOSTINI HT*
*Department of Ophthalmology, Freiburg*

**Purpose** Macular oedema occurs when the blood-retinal barriers (BRB) brake down. The two anatomical structures that prevent the accumulation of extravascular fluid are the retinal blood vessels on the inner side and the retinal pigment epithelium (RPE) as the outer BRB. A variety of disorders can result in macular edema. Possible molecular pathways will be discussed.

**Methods** The change of cellular function with consecutive loss of the BRB can result from direct toxic effects as well as from cytokine mediated regulation. In vitro studies, a variety of animal models and clinical data contribute to the emerging picture of how retinal vascular permeability can be influenced.

**Results** Ischemia and inflammation are the major stimuli to induce macular edema. However, the more we know about the underlying molecular mechanisms the harder it is to distinguish a single cause. In diabetic macular edema for example increased leukostasis is associated with upregulation of ischemia induced permeability factors like VEGF or IGF-1 and hyperglycaemia does not only lead to early pericyte loss but also to activation of the renin-angiotensin system with an increase of Angiotensin II and resulting upregulation of VEGF-A and VEGFR-2. Different mechanisms are involved in macular edema following intraocular surgery with an increase of phospholipase A2 activity due to trauma. Prostaglandins and other lipid derived soluble messengers have a strong impact on the BRB.

**Conclusion** Macular oedema is a sight threatening disorder even in cases with a limited course. A better understanding of the underlying molecular mechanisms will help to further improve the preservation of central vision by specific surgery or drug therapy.

## ▪ 3232
## Laser treatments for macular oedema related to ischemic microangiopathies

*DOSSO A*
*University Eye Clinic, Geneva*

**Purpose** Macular edema is most commonly seen following venous occlusive disease and diabetic retinopathy. A variety of approaches to the treatment of macular edema have been attempted, with a variable degree of success. The place of laser treatment will be discuss.

**Methods** Photocoagulation is a therapeutic technique using a strong light source to coagulate tisssue. Several studies showed that focal laser photocoagulation reduces the risk of vision loss in patients with clinically significant macular oedema.

**Results** A variety of mechanisms may be involved in successful laser photocoagulation for macular oedema including (1) the upregulation or downregulation of chemical factors such as pigment epithelium derived factor (PEDF), vascular endothelial growth factor (VEGF), (2) thermal vascular thrombosis, sclerosis, or leucostasis, (3) alterations in inner and outer blood-retinal barriers, (4) decrease of the oxygen consumption of the outer retina, (5) gliosis.

**Conclusion** Laser treatment remains one of the most effective intervention in the management of macular oedema related to ischemic microangiopathies. New less-invasive photocoagulation strategies (i.e., light laser photocoagulation and subthreshold micropulse diode laser) are emerging.

## ▪ 3233
## The VEGF Inhibition Study in Ocular Neovascularization (VISION) Clinical Trial Group

*ADAMIS T*

**Purpose** To assess the vision benefit of treating early subfoveal choroidal neovascularization secondary to age-related macular degeneration (AMD) with pegaptanib sodium.

**Methods** Exploratory analyses of week 54 vision outcomes (V.I.S.I.O.N. study) of subject subgroups (Groups 1 and 2) with early disease receiving pegaptanib 0.3 mg or sham injections (usual care). Two sets of clinical characteristics typical of early disease defined the subgroups.

**Results** Baseline characteristics were generally well balanced between treatment arms. Pegaptanib responder rates (loss of <15 letters of visual acuity [VA]) were 76% and 80% in Groups 1 and 2, versus 50% and 57% for usual care (pegaptanib relative benefits, 54% and 40%; P-values, 0.03 and 0.05). Compared with subjects assigned to pegaptanib, those in Groups 1 and 2 receiving usual care on average lost 67% and 76% more VA, respectively ($P<0.01$; $P<0.006$). Subjects assigned to usual care were approximately 10 times more likely to experience severe vision loss (Group 1, 29% versus 3%, $P<0.01$). In Group 1, 12% of pegaptanib-treated subjects gained ≥15 letters of VA versus 4% receiving usual care; 20% of Group 2 pegaptanib-treated subjects gained ≥15 letters of VA versus 0% in usual care subjects.

**Conclusion** Early detection and treatment with pegaptanib may result in superior vision outcomes in patients with neovascular AMD.

## ▪ 3234
## Macular oedema of inflammatory origin: anti-TNF treatments

*DE SMET M, REMICADE EUROPEAN STUDY IN CHRONIC UVEITIS RESCU*
*Ophthalmology, University of Amsterdam, Amsterdam*

**Purpose** Anti-TNF is a potent inhibitor of vascular leakage. Therefore, an action on macular oedema may be expected. This particular study was initiated to determine the efficacy of infliximab in the treatment of patients with chronic uveitis, and more specifically its ability to reduce macular oedema.

**Methods** Fluorescein angiograms and when available OCT measurements were analysed between week 0 and 10 in uveitis patients treated as part of the RESCU trial. The analysis of fluoresceins concentrated on changes in the area of fluorescein leakage, and changes in the average greyscale within the area of leakage as measured with the NIH Image program. OCT macular thickness and volume were compared between the two timepoints.

**Results** 49 patients were included in the study. The diagnostic groups included: Behçet's disease (n=9), sarcoidosis (n=5), intermediate uveitis (n=11), birdshot retinochoroiditis (n=10), idiopathic vasculitis (n=7), and sympathetic ophthalmia/Vogt Koyanagi Harada (n=7). By week 10, infliximab was stopped in 4 patients for side effects or reaching an end point. Significant reductions in vitreous haze and improvement in visions were noted over the first 10 weeks. Reduction in macular edema paralleled improvements in vision.

**Conclusion** Anti-TNF therapy appears to be effective in a number of uveitic conditions over the first 10 week period. It also appears to be effective in reducing macular edema in a number of uveitic entities.

■ **3235**

## Vitrectomy for tractional macular oedema

*POURNARAS CJ*
*Department of Ophthalmology, Geneva*

**Purpose** Macular edema is often related to conditions inducing traction on the vitreoretinal interface, most commonly following to intraocular surgery, retinal ischemic microangiopathies, epiretinal membrane traction and posterior segment inflammatory diseases. Among the variety of approaches for the treatment of macular edema, pars plana vitrectomy has also been employed.

**Methods** Macular epiretinal membranes (ERMs) responsible for traction macular edema and decreased vision or metamorphopsia were obtained during vitrectomy for traction-induced diabetic macular oedema (DME), vitreomacular traction syndrome (VMTS) and idiopathic ERM. Electron microscopy, immunohistochemistry and confocal microscopy with antibodies recognizing a-smooth muscle (SM) actin, desmin, TGF-b1, TGF-b receptors (R) I and II, and ED-A FN, were performed. The functional and OCT-evaluated morphologic outcomes of those patients were assessed.

**Results** Traction mechanisms implying a-SM actin-positive myofibroblasts, probably dependent on the concomitant neo-expression of TGF-b1, TGF-bRII and ED-A FN, lying scattered throughout the ERMs of various origins, seem to account for contractile properties of ERM in VMTS, idiopathic ERM and traction-related DME. Macular traction relief leads to impovement of anatomical and functional outcome, although recovery of normal foveal thickness is not achieved in the majority of the cases with ERM. Internal limiting membrane peeling does not affect the functional outcome of idiopathic ERM and DME cases.

**Conclusion** Pars plana vitrectomy is an efficient approach for the treatment of traction-induced macular oedema, although functional improvement is not associated to recovery of normal foveal thickness. Moreover, OCT foveal thickness evaluation alone is not sufficient to predict the final functional results.

## ▪ 3241
## Effect of TSTT on immunological parameters of uveal melanoma

*JAGER MJ, DENNAOUI J, MAAT W, JOURNéE- DE KORVER H, DE WOLFF-ROUENDAAL D, SCHALIJ-DELFOS NE*
*Department of Ophthalmology, Leiden*

**Purpose** In the past, an effect on immunological parameters by hyperthermia and transpupillary thermotherapy has been demonstrated. Therefore it is most likely that transscleral thermotherapy on uveal melanoma may also result in a change of expression of intra- and extracellular proteins. The aim of this study is to determine whether treatment of uveal melanoma by TSTT will lead to a change of expression of the following immunological parameters: heat-shock proteins, S100 and HLA or a change in the presence of macrophages. TSTT was applied with a hot water probe, a laser probe or a combination of both.

**Methods** Experimental TSTT was applied on uveal melanomas prior to enucleation. Expression of heat shock proteins, S100, HLA and macrophage markers were determined on paraffin-embedded tissue from eleven enucleated eyes. The immunological parameters were determined by APAAP immunohistochemistry.

**Results** In uveal melanoma, the level of expression of HSPs varied. S100, HSP90, HSP70, HSP27 and Grp94 not expressed in the TSTT-spots. An upregulated Gp96 expression was observed in the borders of the TSTT spots. Many macrophages were present at the borders of all TSTT-spots in uveal melanomas. In uveal melanoma, the level of expression of HLA-A and HLA-B varied and heat had no clear effect.

**Conclusion** Transscleral thermotherapy has an effect on immunological parameters with local loss of heat shock proteins and S100. This effect may prevent cell protection. The induction of macrophages in the borders indicates that there is an immune reaction against the induced necrosis. It can be concluded that TSTT influences the expression of some immunologically relevant proteins on uveal melanoma.

## ▪ 3242
## Evolution of pigmented episcleral deposits after brachytherapy of uveal melanoma

*TOIVONEN P, KIVELÄ T*
*Department of Ophthalmology, Helsinki University Central Hospital, Helsinki*

**Purpose** To characterize the evolution of pigmented episcleral deposits in eyes with uveal melanoma following brachytherapy and to probe for possible association with melanoma-related mortality.

**Methods** A combined prospective follow-up and retrospective cross-sectional study of 211 patients (M:F 108:103; median age, 61 y; range, 14-88) under review in a national ocular oncology center after ruthenium and iodine plaque therapy (median dose to tumour base, 475 and 392 Gy, respectively) for a uveal melanoma. The location and number of deposits was recorded at the slit lamp during each visit. The association of the deposits with tumour characteristics and survival was analyzed with logistic regression and Kaplan-Meier analysis.

**Results** Median tumour LBD was 12 mm (range, 3-26) and height 5.5 mm (range, 1.5-16.8) at diagnosis, and median reduction in height was 22% and 42% by 6 and 24 months after treatment, respectively. Most deposits appeared within the first 6 months, but the median number slowly increased until 6 to 8 years from therapy. The mean number decreased with increasing distance from tumour center, but deposits were found even on the opposite side. Higher than the median number of deposits was independently associated with plaque diameter (P=.004), ruthenium isotope (P=.02) and tumour regression (P=.05), but the number at 12 and 24 months was not associated with subsequent melanoma-related mortality (P>.40).

**Conclusion** Association of episcleral deposits with plaque size and isotope rather than with tumour size suggests that radiation atrophy of RPE and choroid contributes to them in addition to the regressing tumour. The deposits are not associated with mortality. Knowledge of their benign nature may save patients from inadvertent enucleation.

## ▪ 3243
## Circulating Malignant Cells in Uveal Melanoma

*CALLEJO SA, ANTECKA E, BLANCO P, EDELSTEIN C, BURNIER MN*
*The Henry C Witelson Ocular Pathology Laboratory, Ophthalmology, Montreal*

**Purpose** Despite local tumor control, ~50% of uveal melanoma patients (UMP) will develop METS.This suggest that UM has disseminated by the time of diagnosis & UMP have subclinical metastatic disease that is not initially detected.The purpose of the study is to detect circulating malignant cells(CMC) in UMP & correlate them with tumor size and treatment

**Methods** Prospective clinical study.Date of diagnosis, tumor size, type & date of treatment obtained.Time period between treatment & enrollment(T/E) calculated.Nested RT-PCR used with Tyrosinase(TYR) & Melan A(MLA) as markers.Blood collected every 3 months.In each visit, 20ml of blood subdivided in 10 equal aliquots.In each aliquot TYR & MLA were tested (1visit=10 aliquots=10TYR & 10MLA tests).CMC was correlated with tumor size, treatment, and T/E

**Results** 30 UMP: 22 radiated,4 enucleated,4 observed. 136 visits=1360 aliquots & 2720 RT-PCR.UMP enrolled at time of diagnosis & up to 17 years after diagnosis.11 small size UM, 13 medium, 1 large.No UMP showed clinical evidence of METS.29 of 30 UMP tested + for CMC in at least one visit.119 of 136 +visits recorded (87%) with an average of 2 of 10 aliquots per visit + for MLA & 1 of 10 + for TYR.CMC were detected at time of initial UM diagnosis & at different T/E.Enucleated UMP also tested + for CMC up to 7years following therapy.No correlation between CMC & tumor location/size, treatment or T/E

**Conclusion** The detection of CMC indicates that UM should not be interpreted as a localized disease.CMC were recorded at the time of initial diagnosis & thereafter despite of size of the tumor & treatment.Enucleated patients were + for CMC.The initiation of systemic disease ocurred even before UM diagnosis and CMC were present many years after UM treatment.Systemic therapy is indicated

## ▪ 3244
## New blood markers for metastatic uveal melanoma – a pilot study

*PE'ER J (1), FOLBERG (2), MAJUMDAR D (3), KALICKMAN I (4), BARAK V (4)*
*(1) Ophthalmology, Jerusalem*
*(2) Pathology, Chicago, IL*
*(3) Mathematics, Statistics, and Computer Science, Chicago, IL*
*(4) Immunology Laboratory for Tumor Diagnosis, Jerusalem*

**Purpose** Uveal melanoma is the most common malignant intraocular tumor in adults. Despite treatment, the mortality rate from metastases, usually first to the liver, is very high. The most accepted methods for diagnosing liver metastases is liver imaging and blood tests of liver function. To date no blood marker for metastatic uveal melanoma is available, except for MIA which was tested experimentally. We tested Osteopontin, S-100 and MIA as potential markers for metastatic uveal melanoma.

**Methods** We have amassed over 1000 blood samples of uveal melanoma patients. In this pilot study we examined the serum of 15 patients with proven metastatic uveal melanoma and of 37 patients under follow-up for more than ten years without metastatic disease. Among the 15 patients with metastases, we had serum from 8 patients before and after the diagnosis of metastases. In addition we examined the serum of 30 healthy age- and sex-matched controls. The markers were checked using ELISA analysis.

**Results** The Osteopontin levels in the serum of patient with metastases were almost three times higher than in patients with long follow-up and the control group ($p < 0.001$). There was a significant rise of Osteopontin levels in the 8 patients who developed metastases during the study. The S-100 levels in patients with metastases were approximately twice those of patients with long follow-up and the control group, but there was no statistical significance. There was no difference in the MIA levels among the three groups.

**Conclusion** In this pilot study we found that blood Osteopontin levels can serve as a marker of metastatic disease of uveal melanoma. It is possible that S-100 can also be a marker but this must be tested in a larger number of patients.

## ▪ 3245
## FGFR4 Expression in Choroidal Melanoma

*MADIGAN MC (1), CORNISH EE (1), NATOLI R (2), PROVIS JM (2)*
*(1) Save Sight Institute, University of Sydney, Sydney*
*(2) Research School of Biological Sciences, ANU, Canberra*

**Purpose** Fibroblast growth factors (FGFs) are involved in diverse processes including cell differentiation, angiogenesis and tumour growth. FGFs mediate these biological effects by binding to high-affinity tyrosine-kinase receptors (FGFR1-FGFR5) and extracellular matrix components. Overexpression of FGFR4 has been reported in pancreatic, breast, prostate and pituitary cancer, and in cutaneous melanoma cell lines. In this study we investigated FGFR4 expression in a series of choroidal melanoma specimens, uveal melanoma cell lines and cultured primary choroidal melanocytes.

**Methods** Sections of whole eyes with choroidal melanomas of mixed spindle/epithelioid morphology (n=10) were immunolabelled for FGFR4 using a polyclonal antibody to the carboxy terminus of FGFR4. Antibody binding was visualised using fluorochromes and viewed with confocal microscopy. FGFR4 expression (protein and mRNA) was assessed in uveal melanoma cell lines (OCM-1, OMM-1 and OCM-8) and cultured primary human choroidal melanocytes using immunocytochemistry, Western blots and RT-PCR.

**Results** Cytoplasmic FGFR4 immunoreactivity was observed in tumour cells of primary choroidal melanomas and in uveal melanoma cell lines. Cultured choroidal melanocytes did not show obvious FGFR4 immunoreactivity. Immunoblotting showed a positive band at approximately 120 kDa for uveal melanoma cell lines. FGFR4 mRNA was detected in uveal melanoma cell lines.

**Conclusion** These observations indicate that FGFR4 (and perhaps other FGFRs) may be involved in the growth of choroidal melanoma. The interactions of FGF family members, including FGF-19 (that is highly specifc for FGFR4), and FGRs in choroidal melanoma remain to be fully elucidated.

## ▪ 3246
## Causes and consequences of exudative retinal detachments after protons for uveal melanomas

*GRANGE JD (1), KODJIKIAN L (1), CHAUVEL P (2), GAMBRELLE J (1), ABI AYAD N (1)*
*(1) University Eye Clinic Croix Rousse Hospital, Lyon*
*(2) Biomedical Cyclotron Antoine Laccassagne Anticancer Center, Nice*

**Purpose** The goal of this study is to analyze retinal detachments present after the 24th month following protontherapy for uveal melanomas.They can be present at initial diagnosis or appear later during follow-up . Neovascular glaucoma is frequent and we will try to understand why such situations exist and if survival is influenced .

**Methods** Since june 1991, 450 patients with uveal melanoma were send from Lyon to Nice to be treated with protonbeam ( Biomedical Cyclotron ).A first serie of 226 patients has been analyzed : 9 patients were found with chronic retinal detachment .Mean age was 55 years .2 had no detachment at initial stage , the other 7 had detachment as soon as diagnosis was done .

**Results** There were 8 T2 and 1 T3 melanomas.Significant differences between echographic and intraoperative diameters measurements were seen in all cases except one .A right modelization of the eyeball was done by tomodensitometric slides every mm and a half by radiotherapists in all cases .100% of pseudorecurrences have been observed before the 18th month with temporary increase of tumour volume and then perfect tumour growth control after the 18th month .In 6/9 globes there was an haemorhagic intravitreal or retinal compounent .3 globes out of these 6 had neovascular glaucoma ( 1 enucleation ) .In 3/9 globes there was no haemorhage .2 out of the 3 globes had neovascular glaucoma ( 2 enucleations ).7 patients are alive .

**Conclusion** We observed 4% of recurrent detachment of the retina after protons for uveal melanomas .There is no relation between tumour volume and such chronic detachment .Pseudorecurrence of tumour could be interpreted as necrosis effect .Survival is not affected .

## ▪ 3247
## New iris colour classification and its relationship with uveal melanoma

*MUÍñOS DíAZ Y (1), SAORNIL MA (1, 2), BLANCO MATEOS G (1, 3), MENDEZ MC (1), ALMARAZ A (4), MOREñO MUñOZ MF (4), HINCAPIE J (5)*
*(1) IOBA. Ocular Pathology Laboratory, Valladolid*
*(2) HCUV.Ophthalmology Service, Valladolid*
*(3) IOBA. Orbit an Plastic Surgery Service, Valladolid*
*(4) HCUV. Preventive Medicine Service, Valladolid*
*(5) IOBA. Ocular Oncology Master, Valladolid*

**Purpose** To design a standard, simple, reliable iris color classification.To apply to patients with uveal melanoma.To compare iris color distribution in general population with uveal melanoma patients.To analyze the known prognostic factors according new classification

**Methods** Design classification in 3 colors:Blue-Grey,Hazel-Green and brown-black. Preliminary study had made with 50 digital photos for observers training and to verify reliability.Establishment the distribution in 259 iris photos of voluntary patients made by three observers.Data will be registered in an Access data base.Application of the new classification to uveal melanoma patients form the Ocular Oncology unit in our Institution.Study of the survival and prognostic factors according to the iris color. Statistical treatment through SPSS with percentage and Kappa index and Kaplan-Meier curves

**Results** Classification agreement was 89,6% (0.825 Kappa index). Iris color distribution in the population was 16,3% blue-gray, 55,2% hazel-green and 28,5% brown-black. In patients with uveal melanoma was 16,9% blue-gray, 40% hazel-green and 38,1% brown-black.Blue-gray uveal melanoma group presented significantly smaller survival and an increase of risk of extraocular extension.Not relation was found with other prognostic factors studied (Size, ciliary body involvement, cellular type)

**Conclusion** The established classification is reliable.An inverse proportion in the iris color of patients with uveal melanoma to other published series.The group of blue-grey color is associate to a smaller survival and an increase of risk of extraocular extension.
Partially funded by grant Jose Mª Aguilar Bartolomé

■ 3251

## UBM and in vivo confocal microscopy (IVCM) in wound healing after amniotic membrane transplantation

*NUBILE M, LANZINI M, TOTO L, COSTANTINO O, CAPPONI AL, GAVALAS C, MASTROPASQUA L*
*Ophthalmic Clinic - University "G.d'Annunzio", Chieti-Pescara*

**Purpose** To evaluate confocal microscopic and ultrasound biomicroscopic pattern of wound healing after single and multi-layer amniotic membrane transplantation (AMT)

**Methods** Prospective observational case series. 20 eyes of 20 patients treated by AMT with epithelial side up(multi-layer AMT for deep corneal ulcers, single-layer AMT for chemical burns, peristent epithelial defect or after band keratopahty removal) underwent IVCM (HRT II) and UBM exam at day 1,3,7,15, 30,60,90 and 180 after AMT. Main outcome measures: microscopic AM and corneal layers morphology, integration patterns of AM with corneal tissues.

**Results** Surface epithelial cells of AM persisted visible for an average time of 5 ± 1.1 days, and then underwent progressive degradation, with sparing of subepithelial AM sublayers. For single-layer AMT new corneal epithelium was visible behind residual AM tissue after 18 ± 6.2 days. Following degradation of superficial AM residual tissues occurred within one month in most cases, but appeared longer (up to 3 months) in case of progressive conjunctivalization. Superficial AM layers behaved similarly in multi-layer AMT and a new corneal epithelium, covering the AM tissue filling the ulcer bed, was visible behind residual surface AM tissue after mean time of 21 ± 7.4 days. Deep AM layers were visible in the ulcer bed, under corneal epithelium up to 6 months. UBM evidenced that integration of AM tissues within corneal stroma occurs only in multi-layer AMT leading to a stromal re-thickening during wound-healing.

**Conclusion** IVCM and UBM showed that integration of AM occurs underlying the new corneal epithelium in multi-layer AMT, while superficial or single-layer AMT act promoting epithelization.

■ 3252

## Bves Regulates Corneal Epithelial Monolayer Formation

*CHANG MS (1), BADER DM (2)*
*(1) Department of Ophthalmology and Visual Sciences, Vanderbilt University, Nashville, TN*
*(2) Division of Cardiovascular Medicine, Vanderbilt University, Nashville, TN*

**Purpose** Bves (blood vessel epicardial substance), is a novel adhesion molecule expressed in the developing and adult cornea. Our previous studies indicate Bves is an integral membrane junction protein playing a role in corneal development and regeneration. Evidence is now provided that Bves is critical in the formation and maintenance of the corneal epithelial monolayer.

**Methods** Stably transfected clones over–expressing a full–length(WTX) and a dominant negative truncated Bves (DN) were generated from a human corneal epithelial cell line (HCE). Isolates were analyzed for alterations in junction formation by immunohistochemistry, Western analyses, cellular growth rate, transepithelial electrical resistance (TER), and cellular migration in a cell culture wounding model.

**Results** The DN cells exhibited impaired monolayer formation, altered morphology, and accelerated growth rate. In the wounding model, DN demonstrated rapid but disorganized healing. WTX demonstrated phenotypes essentially opposite that of the DN. WTX formed a organized monolayer with uniformed cells. but exhibited slow wound healing. On Western analysis, ZO-1 and claudin expression were decreased in DN cells indicating tight junction (TJ) formation is altered by Bves disruption. WTX exhibited the highest TER and DN cells the lowest, consistent with altered ZO-1 and claudin expression.

**Conclusion** These results indicate that Bves is critical in the formation of the corneal epithelial monolayer by regulating the initiation and maintenance cell-cell contact in a monolayer. The broader implication is that Bves plays an important role in regulating corneal epithelial cell growth and maintaining the highly organized structure of the human corneal epithelium.

■ 3253

## Pterygium Surgery

*WOOD TO (1, 2), WILLIAMS EE (1), HAMILTON DL (1), WILLIAMS BL (2)*
*(1) Associated Ophthalmic Specialists, Inc., Germantown*
*(2) The University of Tennessee Health Science Center, Memphis*

**Purpose** To determine if a pterygium surgical procedure consisting of minimal conjunctival removal, excision of the hypertrophic subconjunctival fibrovascular tissue, application of mitomycin 0.25 mg/ml for 1 minute combined with temporary nasal tarsorrhaphy, and postoperative dexamethasone/antibiotic drops achieves the following: safely simplifies pterygium removal, controls the early side effects of mitomycin, reduces the rate of recurrence, and lessens the need for conjunctival transplantation.

**Methods** Twenty eyes in 19 patients underwent the procedure with mitomycin; fifteen were primary and 5, recurrent. These were compared to a previous group of 28 eyes in 26 patients that underwent pterygium/tarsorrhaphy surgery without mitomycin; twenty had primary and 8 had recurrent pterygia. Postoperatively, all eyes in both groups were treated with dexamethasone/antibiotic drops.

**Results** In the mitomycin group, with an average follow-up of 12.1 months, 19 eyes healed uneventfully; there have been no recurrences. The non-mitomycin group, with an average follow-up of 42.6 months, has had 9 (32%) recurrences; four required a second procedure. Recurrence was significantly lower in the mitomycin group (P = .006). Conjunctival healing, as reflected in the time from surgery until tarsorrhaphy opening, was significantly delayed in the mitomycin group, 36.7 vs. 17 days (P = .001). The delay in conjunctival healing may explain the complications associated with the use of mitomycin in pterygium surgery.

**Conclusion** Minimal conjunctival removal, extensive fibrovascular tissue excision, 1 minute application of mitomycin 0.25 mg/ml, temporary nasal tarsorrhaphy, and frequent postoperative dexamethasone/antibiotic drops provided a safe and successful approach to pterygium management, in this series.

■ 3254

## Are rabbit bone marrow–derived cells able to contribute to corneal epithelial repair?

*GUEUDRY J (1), LAMACZ M (2), VANNIER J-P (2), DUCLOS C (3), METAYER J (3), BRASSEUR G (1), MURAINE M (1)*
*(1) Ophtalmology, Rouen*
*(2) MERCI Laboratory, Rouen*
*(3) Pathology, Rouen*

**Purpose** Autologous stem cell transplantation for total limbal stem cell deficiency is preferable to avoid allograft rejection. Recent studies have shown that bone marrow-derived stem cells were able to differentiate into several lineages of cells. Therefore we explored their potential to differentiate into corneal epithelial cells in vitro and in vivo.

**Methods** Mesenchymal stem cells (MSCs) were obtained from rabbit bone marrow. MSCs multipotency was indirectly proved by being induced to differentiate into chondrocytes, osteoblasts and adipocytes. MSCs were cultured for 15 days and then were expanded on intact or denuded amniotic membrane (AM). Histologic analysis was performed with Hematoxylin-Eosin-Safran staining (HES). Markers for epithelial differentiation were analysed using immunohistochemistry. Stem cells on AM were labelled with iron oxide and autologous transplanted onto a rabbit model of stem cells deficiency. Animals were sacrificed 1 and 2 weeks after and dissected corneas were analysed with HES and Prussian Blue staining.

**Results** Confluent cultured cells were established on AM. Cells were partially stratified in two to four layers and fairly attached to the underlying basement membrane. Immunochemistry results were negative for epithelial markers. Some grafted cells persisted on the ocular surface 15 days after transplantation. Autologous transplantation failed to reconstruct transparent cornea in rabbits.

**Conclusion** MSCs cultured on AM can organise in multilayer fashion under specific conditions. Autologous transplantion of cultivated MSCs is feasible by using AM as a basement membrane and as a carrier. However, epithelial differentiation in vitro has still to be proved.

## ▪ 3255
## Back to Basics-a simple technique for repair of Descemets membrane tear

*HUNTBACH JA (1), RAUF A (2)*
*(1) Queens Medical Centre, Nottingham*
*(2) Queens Hospital, Burton-on-Trent*

**Purpose** To demonstrate a simple and effective method for the repair of a Descemets membrane tear.

**Methods** A 78 year old female underwent cataract surgery complicated by post-operative corneal decompensation, subsequently found to have been caused by a tear in Descemets membrane. Surgical options for reapproximating the membrane include transcorneal mattress sutures with intracameral injection of air, or more recently intracameral injection of slow-reabsorbing gases such as SF6 and C3F8. No evidence-based study exists at present to assess one technique as superior to another. We adopted a basic approach using healon to unroll the membrane followed by injection of air alone. The patient postured supine for one hour and was thereafter discharged. Pre- and post-operative anterior segment photos were taken together with a video of the surgical technique.

**Results** The results were excellent. Initially the patient had severe corneal oedema with a visual acuity of only 0.5/60. Following surgery, there was a rapid improvement of visual acuity which continued over a two week period to give a final best corrected acuity of 6/12 with a clear cornea.

**Conclusion** We illustrate a simple and successful technique for repair of Descemet membrane tear. We suggest this approach should be assessed further as first line treatment as the results are comparable to those of more complex techniques.

## ▪ 3256 / 233
## Corneal wound healing after intrastromal femtosecond laser keratectomy

*MELTENDORF C (1), BURBACH GJ (2), BüHREN J (1), OHRLOFF C (1), DELLER T (2)*
*(1) Department of Ophthalmology, Johann Wolfgang Goethe-University, Frankfurt am Main*
*(2) Institute of Clinical Neuroanatomy, Johann Wolfgang Goethe-University, Frankfurt am Main*

**Purpose** The purpose of this project was to examine the corneal repair response after intrastromal femtosecond keratectomy which is characterized by the absence of epithelial damage after surgery.

**Methods** Twelve rabbits underwent monocular intrastromal keratectomy at a corneal depth of 200 µm using a femtosecond-laser. Follow-up examinations were performed 1, 3, 7, and 28 days after surgery. Corneas were evaluated using slit lamp and in vivo confocal microscopy. Immunofluorescence microscopy was used to localize fibronectin and tenascin C as markers of early stromal wound healing. Nuclear DNA fragmentation was detected with the TUNEL assay. Anti-α-smooth muscle actin was used to determine if myofibroblasts were present.

**Results** On slit-lamp examination, the corneas were transparent 1 day after surgery. Keratectomy was detectable as a narrow opaque band that was still visible after 28 days. After 1 day, TUNEL-staining revealed a broad zone of TUNEL-positive keratocytes. Immunofluorescence studies disclosed the deposition of fibronectin and tenascin C in the same area already 1 day after surgery. In vivo confocal microscopy showed a regular pattern of highly reflective laser spots and an irregular network of brightly reflecting keratocytes within the keratectomy zone after 1 day. After 28 days, this zone showed unstructured acellular areas with increased reflectivity. No α-smooth muscle actin was detected at any time point.

**Conclusion** Keratocyte apoptosis occurs at the zone of intrastromal keratectomy despite the absence of a concomitant epithelial debridement. The lack of myofibroblast formation indicates that the integrity of the basement membrane might be a crucial factor in limiting the fibrotic repair response.

## ▪ 3257 / 234
## NGF treatment and corneal wound healing

*BLANCO-MEZQUITA JT (1), MERAYO-LLOVES J (1), MARTÍNEZ-GARCÍA C (2), MAR-SARDAÑA S (3), MARTÍNEZ-OSORIO H (1), TORRES R (1), GONZÁLEZ-PARRA M (1), BONINI S (4), LAMBIASE A (4)*
*(1) IOBA, Valladolid*
*(2) Cell Biology, Valladolid*
*(3) Optics and Applied Physics, Valladolid*
*(4) Laboratory of Ophthalmology, Rome*

**Purpose** The purpose of this research is to evaluate the effectiveness of topical administration of murine Nerve Growth Factor in the wound healing process after refractive surgery

**Methods** Iber Braun hens underwent PRK were divided into different groups treated with topical administration of 0.2% murine Nerve Growth Factor (NGF) group A, Balanced Salt Solution (BSS) group B and group C received no treatment. Fluorescein staining, clinical follow up of haze, pachymetry, and both transmitance and scattering measurements were taken. Eyes were exenterated at sequential time points and fixed in 10% buffered formalin. Sections were stained with H-E. TUNEL technique was used for apoptosis detection. Antibodies anti-BrdU were used for cell proliferation. Antibodies anti-⊠-SMA were used for myofibroblast differentiation. TrkA receptors and cytochrome c were detected by immunohistochemestry.

**Results** There was not difference in the recovery of the epithelium integrity between groups. Differences in earlier apoptosis , cell proliferation, cell differentiation and cytocrome c location were found between groups. Statistically significant differences between the appearance and disappearance of the haze, pachymetry, transmitance and scattering were found.TrkA receptors was found in the epithelium, endothelium ans keratocytes in all the corneas

**Conclusion** In normal corneas, NGF has not effect in the recovery of epithelium integrity Stromal apoptosis, proliferation and myofibroblast differentiation were modulated by NGFTrkA receptors are present in the cornea of hens which remarks that this is a very good animal model to study corneal wound healing after refractive surgery

■ 3261

## Oxidative stress from UVR in the lens

*SÖDERBERG PG (1, 2), LöFGREN S (3, 1), AYALA M (4), DONG X (1), MODY V (1), KAKAR M (1), MEYER L (1)*
*(1) Section of Ophthalmology and Vision, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm*
*(2) Department of Biomedical Engineering, University of Miami, Miami*
*(3) Department of Veterinary and Biomedical Sciences, University of Nebraska-Lincoln, Lincolm*
*(4) Department of Ophthalmology, Örebro University Hospital, Örebro*

**Purpose** To investigate species differences in oxidative stress from UVR in the lens

**Methods** Albino Sprague Dawley rats, Brown Norwegian pigment rat, pigmented Guinea pigs and pigmented C-57 mice were experimentally in vivo exposed to UVR in the 300 nm wavelength region. A predetermined time after the exposure, the animals were sacrificed and the lenses were removed for macroscopical imaging and measurement of forward light scattering.

**Results** In the albino Sprague Dawley rat, the intensity of forward light scattering increases exponentially declining during a week and then remains constant. In the pigmented Brown Norwegian rat, the light scattering increases transiently with a peak that is higher and that comes earlier the higher the dose. In the Guinea pig and the C-57 mice, the light scattering reaches a maximum already 1 day after exposure and then remains constant. The sensitivity expressed as the inverse of the threshold dose for damage increases from Guinea pig to Brown Norwegian rat, to albino pigmented rat to pigmented C-57 mice.

**Conclusion** There is a marked species variation in oxidative stress from UVR in the lens.

■ 3262

## Dietary carotenoids and polyphenols in cataract prevention

*TREVITHICK JR (1), SUTTON-TREVITHICK CC (2), COLLINS MM (1), DZIALOSZYNSKI TM (1)*
*(1) School of Kinesiiology, Univ. W. Ontario, London, Ontario*
*(2) Chemistry, UCLA, Los Angeles, CA*

**Purpose** To investigate the possibility of an antioxidant mechanism for reduction of cataract risk in people consuming dietary carotenoids and polyphenols.

**Methods** Antioxidant activity of a number of dietary components and beverages was tested using a luminescent assay and ESR spin-trapping.

**Results** Antioxidants can prevent mitochondrial damage leading to calcium release, calpain activation, spectrin/fodrin proteolysis, and lens fibre cell globulization resulting in opacification. Cataract risk reduction is associated with moderate consumption of alcoholic beverages, as well as vitamins C and E and the carotenoid lutein in the diet. Beer appears to be the major source of antioxidants in the American diet. Alcoholic beverage consumption decreased cataract risk at one drink per day, but increased it at three drinks per day. Consistent with this, the plasma of volunteers was antioxidant after one standard drink of beer or wine, but became pro-oxidant after three drinks. Polyphenols, carotenoids and flavonols, are the major antioxidants which dietary components and alcoholic beverages contain. Carotenoids were previously not believed to have antioxidant activity, but our ESR studies indicate that they act similarly to vitamin C and are able to destroy both superoxide and hydroxyl radicals.

**Conclusion** Dietary antioxidant carotenoids and polyphenols as well as vitamin C, contained in foods and beverages, can protect against cataract. Further studies are needed to clarify the exact role of antioxidants in reducing risk of cataract, but protection of lens equatorial fibre cell mitochondria appears to be a likely mechanism for cortical cataract risk reduction.

■ 3263

## Cross-Linking of Oxidatively-Modified Crystallin Fragments in vivo

*SRIVASTAVA OP*

**ABSTRACT NOT PROVIDED**

■ 3264

## Extrinsic use of intrinsic antioxidants for prevention of cataracts

*HEGDE KR (1), VARMA SD (1, 2)*
*(1) Ophthalmology, Baltimore*
*(2) Biochemistry, Baltimore*

**Purpose** Since cataract is a progressive disease, treatment aimed at its prevention or attenuation by antioxidants of exogenous origin such as some of those derived phytochemically may not be as suitable as desired for continued use because of their toxicity. We have therefore explored the feasibility of using endogenously derived scavengers of ROS especially those that can also act as metabolic agonists.

**Methods** Cataracts were induced in neonatal rat pups by I. P. administration of sodium selenite in the dose of 0.5µmoles/animal. α-ketoglutarate was used as an ROS scavenger. Inhibition of cataractogenesis by this compound was studied by its I. P. administration in the dose of 0.13 millimoles/animal/day started 2 days prior to selenite injection and continued for 21 days later. Cataractogenesis was monitored ophthalmoscopically as well as biochemically by determination of ATP and GSH.

**Results** Administration of sodium selenite resulted in reproducible induction of cataracts within one week after its injection. The % of eyes with cataracts in these pups was 83% in the untreated selenite group as compared to ~23% in the α-KG + selenite group. Cataract formation was associated with a significant decrease in the level of ATP and GSH, the decrease being prevented substantially by α-KG treatment

**Conclusion** That the cataract preventive effect of α-KG is related to its antioxidant and metabolic effects is evident by the higher level of GSH and ATP in the lenses of rat pups treated with this agent. The results suggest that intrinsic compounds with antioxidant activity could have clinical usefulness in the attenuation and prevention of senile cataracts.

■ 3265

## Vitamin E in protection against cataract

*AYALA M (1, 2), SÖDERBERG PG (2)*
*(1) Eye department, Örebro*
*(2) St. Erik's Eye Hospital, Karolinska Institut, Stockholm*

**Purpose** Purpose: to investigate if vitamin-E (α-tocopherol) protects against ultraviolet radiation (UVR) induced cataract

**Methods** Methods: 40 Sprague Dawley rats were divided into two groups with 20 animals in each. One group was fed with vitamin-E: 100 IU α-tocopherol/day once a day during 4 weeks and the other group was used as control. Both groups were unilaterally exposed to 8 kJ/m² UVR. One week after the exposure the rats were sacrificed, both lenses were removed, and forward light scattering was measured. Vitamin-E concentration in the lenses was measured by high performance liquid chromatography (HPLC)

**Results** Results: Exposed lenses in the vitamin-E group showed superficial cataract. Meanwhile lenses in the control group (without vitamin E) showed superficial, central and equatorial cataracts as same as vacuoles. Forward light scattering measurement in the control group was higher than in the vitamin-E treated group. Vitamin E levels in the lens were significant higher in the treated group than in the control group.

**Conclusion** Conclusions: in the rats an antioxidant like the vitamin-E seems to protect the lens against UVR-induced cataract. Discussion: there are still controversial points to be discussed: per-oral treatment versus eye-drops; vitamin E stability and penetration in the ocular tissues; side effects; combination with other antioxidants (vitamin C, pyruvate), etc.

■ 3266

## Induction of Glyoxalase I Fails to Prevent Methylglyoxal Accumulation in Diabetic Mouse Lenses

*NAGARAJ R, STANISZEWSKA M*
*Ophthalmology, Cleveland*

**Purpose** Methylglyoxal (MGO) is a highly reactive protein modifier present in the lens. It is produced during metabolism and by oxidative degradation of glycated products. Glyoxalase system consisting of glyoxalase I and glyoxalase II metabolizes MGO. In this study we studied the effect of hyperglycemia on glyoxalase I and its role in MGO metabolism in lens epithelial cells and lenses of diabetic mice

**Methods** Mouse lens epithelial cells were cultured for 7 days in the presence of 5 or 25 mM D-glucose or 25 mM L-glucose (osmotic control). Glyoxalase I activity was measured spectrophotometrically and its mRNA content was measured by quantitative PCR. We also measured MGO and reduced GSH in cell lysates. Similar measurements were done in lenses of diabetic mice.

**Results** Glyoxalase I activity in 25 mM D-glucose treated cells was significantly higher when compared cells treated with 5 mM D-glucose or 25 mM L-glucose. Glyoxalase I mRNA content was also significantly higher ($P<0.01$) in 25 mM D-glucose treated cells. MGO levels were significantly higher ($P=0.007$) in cells grown in 25 mM D-glucose when compared cells grown in 5 mM D-glucose or 25 mM L-glucose. GSH levels were considerably reduced in cells cultured with 25 mM D-glucose. In diabetic mouse lenses, glyoxalase I activity and mRNA content were significantly higher than in control animals (glyoxalase I: control-137.8±1.11; Diab-161.1±24.2, $P=0.003$; fold change in glyoxalase I mRNA in diabetic lenses over controls-1.31±0.2).

**Conclusion** Increased oxidative stress resulting in a loss of GSH in diabetic lenses may compromise MGO metabolism by glyoxalase. Higher production of MGO, in spite of elevated glyoxalase I might lead to chemical modification of proteins by MGO in the diabetic lens.

■ **3311**

## Functional magnetic resonance imaging of the cerebral response to visual stimulation in medically unexplained visual loss

*PLANT GT, WERRING DJ, WESTON L, BULLMORE ET, RON MA*
*Department of Neuroinflammation, Institute of Neurology, London*

**Purpose** Medically unexplained visual loss occurs in 1 to 5% of patients attending ophthalmology clinics and for many it runs a chronic course. A psychogenic aetiology is presumed in such cases, but little is known about the underlying neural mechanisms. Recent studies have established the value of functional magnetic resonance imaging (fMRI) in understanding the mechanisms of unexplained motor and sensory symptoms. The purpose of this study was to use a similar strategy (fMRI) to evaluate the cerebral responses to visual stimulation in a group of patients with medically unexplained visual loss, in an attempt to determine the underlying neural mechanisms. METHOD: Brain activation induced by periodic (monocular) 8 Hz visual stimulation was detected by fMRI in five patients with unexplained visual loss who fulfilled DSM-IV criteria for conversion disorder, and seven normal volunteers. Between-group differences in mean power of activation were estimated by fitting a one-way analysis of variance (ANOVA) model at each intracerebral voxel in standard space. RESULTS: Compared with controls, patients showed reduced activation in visual cortices, but increased activation in left inferior frontal cortex, left insula-claustrum, bilateral striatum and thalami, left limbic structures, and left posterior cingulate cortex. CONCLUSIONS: This preliminary study has identified novel neural correlates in patients with unexplained visual loss. The abnormal pattern of activation may reflect inhibition of primary visual cortex or a shift towards non-conscious (implicit) processing.

■ **3312**

## Emotional attributes of the image. Neural mechanisms involved in their processing, and clinical implications

*SAFRAN AB*
*Ophthalmology Clinic, University Hospitals, Geneva*

**Purpose** In recent years, studies on the processing of image emotional features contributed to elaborate new concepts on image analysis and to redefine limits of visual consciousness. They have significant implications for clinical practice, including in everyday ophthalmologic work-up. Of special importance are the findings that image emotional features are vehiculated to amygdaloidal complex (the main structure involved in emotion processing) along two distinct pathways. One of them - cortical - participates to conscious visual perception whereas the other - subcortical - contributes to non-conscious visual processing. Subcortical route to amygdaloidal complex might be selectively tuned for low spatial frequency visual information, whereas visual inputs to the amygdaloidal complex via the consciously mediated cortical pathway might rely on high spatial frequency information.Inactivation of structures involved in conscious vision does not preclude emotional response to apparently unperceived stimuli. Thus, under condition of binocular rivalry, amygdaloidal complex continues to encode affective information from face stimuli that are not consciously perceived. Moreover, patients with occipital lesions have demonstrated increased activity in the amygdaloidal complex and extra-striate cortex following presentation of affective facial expressions in the "blind hemifield". Some controversy however still exists over the effects of emotional information vehiculated by unconscious stimuli. Modulation of perception by emotion has also been emphasized, suggesting that sensory-processing impairments might arise in affective conditions.

■ **3313**

## Out-of-body experience and autoscopy of neurological origin. A visual illusion of one's own-body

*LANDIS T*
*Neurology Clinic, University Hospitals, Geneva*

**Purpose** Autoscopic phenomena such as out-of-body experience (OBE) and autoscopy (AS) are complex visual illusions. During OBE the subject seems to be awake and to see his body and the world from a location outside the physical body, whereas AS is characterized by the experience of seeing one's body in extrapersonal space. During both experiences the subject sees himself as a part of the extrapersonal world. During OBE the subject "sees" from a location other than his physical body, whereas during AS he remains within the boundaries of his physical body.We will describe clinical correlates of OBE and AS in neurological patients and provide evidence that both experiences share important central mechanisms such as vestibular sensations and visual body-part illusions. Brain dysfunction was localized to the temporo-parietal junction (TPJ). We speculate that OBE and AS are due to ambiguous input from different sensory systems that are important for the creation of central representation(s) of one's own body. We suggest that OBE and AS are caused by a failure to integrate proprioceptive, tactile, and visual information with respect to one's own body (disintegration in personal space) and by a vestibular dysfunction leading to an additional disintegration between personal (vestibular) space and extrapersonal (visual) space. It is argued that both are due to paroxysmal cerebral dysfunction at the TPJ in a state of partially and briefly impaired consciousness.

## ▪ 3321
## The role of PITX2 in Primary Congenital Glaucoma

*IDREES F (1), FRASER S (2, 3), RUTLAND P (4), STOILOV I (5), SARFARAZI M (5), SOWDEN J (1), KHAW P T (6)*
*(1) Developmental Biology Unit, Institute of Child Health, UCL, London*
*(2) Glaucoma Unit, Sunderland Eye Infirmary, Sunderland*
*(3) Moorfields Eye Hospital, London*
*(4) Clinical and Molecular Genetics Unit, Institute of Child Health, UCL, London*
*(5) Molecular Ophthalmic Genetics Laboratory, Farmington, CT*
*(6) Department of Glaucoma, Moorfields Eye Hospital, London*

**Purpose** To determine the role of PITX2 mutations as a cause of primary congenital glaucoma

**Methods** Patients were recruited in to the study with a diagnosis of primary congenital glaucoma (PCG). This diagnosis was considered if the patient had high intraocular pressures before the age of 5 with clinical findings consistent with glaucoma in at least one affected eye such as breaks in Descemet's membrane, enlarged cornea or buphthalmos. Those patients who had anterior segment developmental anomalies were excluded. Genomic DNA was extracted using standard protocols from whole blood. Mutation screening of the coding region of the CYP1B1 gene including a small region of the 5' and 3' UTR was performed by single-strand conformation polymorphism analysis (SSCP) and direct sequencing of PCR products in both forward and reverse directions. DNA samples of 30 patients with a clinical diagnosis of PCG found to have no mutations in the coding region of CYP1B1 were then analysed for mutations in the PITX2 gene. PCR amplification of the coding region of the PITX2 gene including the intron-exon boundary was performed and automated direct sequencing using the MegaBACE analyser in forward and reverse directions.

**Results** No PITX2 mutations were found in 30 PCG patients

**Conclusion** This is the first reported study to investigate the role of PITX2 mutations in PCG. The primary molecular defect underlying the majority of PCG cases is known to be due mutations in the enzyme CYP1B1. PITX2 mutations have been shown to have a vital role in the development of the anterior chamber including structures of the angle. Our study shows that there is no evidence that PITX2 mutations cause PCG. Genetic testing for PITX2 mutations in PCG is unlikely to be of benefit.

## ▪ 3322 / 339
## A new scoring system for giant cell arteritis (GCA) - Can the result of a temporal artery biopsy (TAB) be predicted?

*NESTEL AR, WHITTAKER KW, GIBSON RA, ENOCH BE*
*North Devon District Hospital, Department of Ophthalmology, Barnstaple, Devon*

**Purpose** To establish a new test for the diagnosis of GCA based on a clinical scoring system and determine its predictive accuracy relative to the outcome of TAB

**Methods** A score chart was devised, with each symptom and sign being allotted a numerical value, ranging from -2 to +3, according to its significance. The numerical values were derived from an extensive literature review and fine-tuned based upon an a initial review of 20 consecutive cases of suspected GCA in whom a biopsy had been performed. At a score of > or = 6 the test was considered positive, at a score of <6 the test was considered negative (- the hypothesis being that a greater proportion of patients with GCA would have scores of > or = 6 compared with patients without GCA). A blind study was then conducted on further 35 consecutive cases in order to determine the test's predictive accuracy.

**Results** The test had a sensitivity of 100%, a specificity of 90 %, a negative predictive value of 100% and a positive predictive value was 50% ($p < 0.01$).

**Conclusion** By virtue of its high sensitivity, the scoring system appears to be a very 'safe' test, successfully identifying all cases of biopsy-proven GCA. This is critical given the potentially devastating nature of the disease. Its excellent negative predictive value was such that, had it been applied prospectively in this series, 82% of TAB could have been avoided. Taken together these results suggest that the scoring system could provide an effective predictive aid. A larger prospective study may be helpful in further establishing the clinical role of such a scoring system.

## ▪ 3323 / 340
## Intraocular Pressure Lowering effect of dorzolamide/timolol fixed combination in non-responder glaucoma patients to prostaglandin analogues/prostamides

*MARTÍNEZ-GARCÍA A, SÁNCHEZ-SALORIO M*
*Instituto Galego de Oftalmoloxía, Santiago de Compostela*

**Purpose** Purpose: To evaluate the effect of the dorzolamide/timolol fixed combination (DTFC) in non-responder glaucoma patients to prostaglandin analogues/prostamides.

**Methods** Methods: 21 patients (1 eye from each) with open-angle or capsular glaucoma were included in this retrospective observational cohort study. 119 consecutive glaucoma patients treated with prostaglandin analogues/prostamides were screened, between June 2003 and December 2004, 21 patients (17.6%) were considered Non-responder. Non-responder was defined as an intraocular pressure (IOP) lowering effect less than 15% compared with baseline measurement. IOP was measured at 8 AM, 10 AM, 12 AM, 2 PM, 4 PM, 6 PM, 8 PM and 10 PM at baseline and at the end of each treatment period.

**Results** Results: At baseline the Highest IOP (mean (SD)) was 27.67 (3.26) mm Hg at 12AM and the lowest IOP was 20.67 (1.83) mm Hg at 4 PM. The IOP was higher at 4PM ($p=0.035$) after treatment with prostaglandin analogues/prostamides compared with baseline measurement. The IOP was lower ($p<0.0001$) in all IOP-time point measured after treatment with the DTFC compared with prostaglandin analogues/prostamides treatment phase.

**Conclusion** Conclusion: DTFC showed a good IOP lowering effect in Non-responder prostaglandin analogues/prostamides glaucoma patients.

## ▪ 3324
## A randomised, prospective study comparing 90°, 180° and 360° Selective Laser Trabeculoplasty with Latanoprost 0.005% for the control of intraocular pressure in Ocular Hypertension and Open Angle Glaucoma

*JONES S, O'BRART DPS*
*Department of Ophthalmology, St Thomas' Hospital, London*

**Purpose** To compare 90°, 180° and 360° Selective Laser Trabeculoplasty (SLT 532nm Nd:YAG laser) with Latanoprost 0.005% for the control of intraocular pressure in ocular hypertension (OHT) and open angle glaucoma (OAG).

**Methods** Prospective, randomised clinical trial in two centres. One hundred and sixty seven patients with either OHT or OAG were randomised to receive either 90°, 180° and 360° SLT or Latanoprost 0.005% at night and were evaluated at 1 hour, 1 day, 1 week and 1, 3, 6 and 12 months.

**Results** Mean follow-up was 10.3 months (range 1 to 12 months). Success rates defined in terms of both a 20% or greater and a 30% or greater IOP reduction from baseline measurements with no additional anti-glaucomatous interventions were better with Latanoprost compared to 90° ($p<0.001$) and 180° SLT ($p<0.02$) treatments. Differences in success rates between Latanoprost and 360° SLT did not reach statistical significance ($p<0.5$). Success rates were greater with 180° and 360° compared to 90° SLT ($p<0.05$). With 360° SLT, 82% of eyes achieved a >20% IOP reduction and 59% a >30% reduction from baseline. There were no differences with regards to age, sex, race, pre-treatment IOP, OHT versus OAG, laser power settings and total laser energy delivered between eyes which responded, in terms of a >20% and a >30% IOP reduction, and those that did not respond with 180° and 360° SLT treatments.

**Conclusion** Success rates were higher with Latanoprost 0.005% ON compared to 90° and 180° SLT treatments. The 360° SLT appears to be an effective treatment modality with approximately 60% of eyes achieving an IOP reduction of 30% or more.

## ▪ 3325
## Safe-Trabeculectomy Technique: long-term outcome

*ZEYEN T (1), GILLIS A (2), LAFAUT AS (1), STALMANS I (1)*
*(1) University Hospital, Dept. Ophthalmology, Leuven*
*(2) Middelheim Hospital, Dept. Ophthalmology, Antwerp*

**Purpose** To assess the long-term outcome of a new trabeculectomy technique.

**Methods** Trabeculectomy was performed using a fornix-based conjunctival flap, an anterior chamber maintainer, a standardized punch technique, and a combination of adjustable and releasable sutures in 56 eyes of 53 patients. The main outcome measures were the postoperative intra-ocular pressure (IOP) and the frequency of early postoperative complications . The mean follow-up time was 15.7 (range 12-21) months.

**Results** The mean pre- and postoperative IOP at 12 months were 21.2 +/- 6 and 12.8 +/- 3.0 mmHg, respectively. All patients had an IOP of <21 mmHg, 90.9% had an IOP <18 and 61.4% had an IOP <14mmHg. Postoperative complications were infrequent: flat anterior chamber (1.8%), bleb leakage (0%) or hypotony (1.5%) beyond 3 weeks, or choroidal detachment at any timepoint (8.9%).

**Conclusion** This novel trabeculectomy method offers the possibility to tailor the IOP postoperatively with a minimum of postoperative complications and excellent long-term IOP control.

## ▪ 3326 / 341
## Safety and efficacy of the Optonol DS version microtube implanted during deep sclerectomy in POAG patients: preliminary results

*POURNARAS J-A C, MERMOUD A*
*Jules Gonin Eye Hospital, Lausanne*

**Purpose** To assess the safety and efficacy of the Optonol DS version microtube implanted during deep sclerectomy in POAG patients.

**Methods** This non-randomized, prospective pilot study include 5 POAG patients (6 eyes) requiring filtering surgery. Deep sclerectomy was performed and the miniature implant was inserted at the anterior part of the deep sclerectomy into the anterior chamber. The complete eye evaluation was performed before and after surgery during 6 months. UBM were also performed.

**Results** At the sixth month, the mean postoperative intraocular pressure was significantly reduced from 21.14 ± 2.41 mmHg to 12 ± 2.7 mmHg (p=0.0019; n=6). All implants were correctly implanted, as shown by the UBM.

**Conclusion** Optonol DS version microtube inserted during deep sclerectomy is well tolerated and significantly reduces the intraocular pressure at the sixth month without any notable complication.

## ▪ 3327
## Trabeculectomy with Mitomycin C – do we need to work harder in the African-Caribbean population?

*SHAH P, SII F, LOCKWOOD A*
*Ophthalmology, Birmingham*

**Purpose** To compare the efficacy of trabeculectomy with mitomycin C in the African-Caribbean population with the Caucasian population at 1 year and to evaluate the predictive power of 1 year results of long-term success.

**Methods** This is a substudy of Phase 3 of the Birmingham ReGAE (Research into Glaucoma and Ethnicity) Project, an open, prospective, consecutive case series of patients who had undergone trabeculectomy with mitomycin C. All eyes of patients of African-Caribbean (AFC) and Caucasian ethnic background with hypertensive glaucoma were included.

**Results** Of 138 eyes (24.6% AFCs, 75.4% Caucasians), 101 eyes (25.7% AFCs, 74.3% Caucasians) had a minimum of 1-year follow-up. At 1 year, a similar proportion of eyes achieved IOP ≤ 21 mm Hg (92.3% AFCs vs 89.3% Caucasians) but fewer AFC eyes achieved IOP ≤ 14 mm Hg than Caucasian eyes (61.5% vs 74.7%). The proportion of eyes with 30% IOP reduction were similar between the 2 groups (73.1% vs 72.0%) but a lower mean IOP reduction in the AFC group (36.3% vs 46.8%). A larger proportion of AFC eyes required anti-glaucoma medication postoperatively (23.1% vs 8.0%) and a higher mean of number of topical medications (0.35 vs 0.11). Similarly, a larger proportion of these eyes required bleb needling revision with 5FU (26.9% vs 20.0%). The rates of failure were 3.8% in the AFC group and 5.3% in the Caucasian group. These 1-year results will be compared with the results of the last follow-up.

**Conclusion** In trabeculectomy with mitomycin C, AFC eyes required more postoperative medical and surgical interventions than Caucasian eyes to achieve significant IOP reductions. Achieving low postoperative target IOP (≤ 14 mm Hg) is more difficult in the AFC patients. The reasons for this will be discussed.

## ▪ 3328
## The use of fibrin glue to secure donor lamellar scleral transplantation in extensive scleral reconstruction in juvenile idiopathic arthritis associated scleromalacia.

*LOCKWOOD A, SII F, SHAH P*
*Ophthalmology, Birmingham*

**Purpose** This video demonstrates the technique of scleral reconstruction in a 23-year-old female with extensive juvenile idiopathic arthritis associated scleromalacia and refractory glaucoma in her only eye (maximal intraocular pressure 42 mm Hg).

**Methods** As part of her planned glaucoma surgery she needed scleral reconstruction of the superior scleral tissue (120°) where there was ectactic choroidal herniation prior to a planned Baerveldt drainage tube insertion.

**Results** The technique of donor lamellar scleral transplantation secured with 10/0 nylon mattress sutures and fibrin glue is shown in this video.

**Conclusion** The video provides expert tips on surgical minutiae in this technique.

## ▪ 3331
## Methods of Evaluation of Optic Nerve Head Circulation

*POURNARAS CJ*
*Department of Ophthalmology, Geneva*

**Purpose** The presence of a coupling between neural function, metabolism and blood flow at the optic nerve head (ONH) has led to investigations of blood flow in this tissue aimed at gaining a better understanding of ischemic conditions. This paper will discuss the applications of methods investigating this flow and also the ONH tissue partial pressure of O2 (PO2).

**Methods** Local laser Doppler flowmetry (LDF) and PO2 measurements with oxygen sensitive micro-electrodes, both performed in the tissue of the ONH, were used to assess blood flow and PO2 changes in experimental animals in response to various physiological stimuli. LDF was also used in clinical investigations of the ONH hemodynamics.

**Results** In normal eyes, ONH blood flow remains constant under a broad range of mean perfusion pressure (PPm) variations. Similarly, ONH blood flow is regulated at constant values during PPm increases in eyes with normal tension glaucoma. Physiological stimuli, such as variations of PPm, of blood PaO2 (hyperoxia and hypoxia), do not induce a significant modification of PO2 values in intervascular areas of the optic disk or at various depths within the ONH. The regulation in the ONH during PaO2 variations is achieved by an adaptation of the blood flow probably through local regulatory mechanisms affecting the tone of the capillaries (relaxation or contraction of their pericytes). However, during hypoxia, regulation of tissue PO2 was absent at deeper ONH layers close to the border of the ONH near the choroid.

**Conclusion** Experimental and clinical data suggest that LDF and PO2 measurements are useful techniques to assess ONH blood flow and PO2 responses to a variety of physiological stimuli.

## ▪ 3332
## Origin of optic disc near-infrared reflectance changes induced by light stimulation.

*RIVA CE (1), LOGEAN E (2), FALSINI B (3)*
*(1) Oftalmologia, Università degli Study di Bologna, Bologna*
*(2) Applied Optics Group, National University of Ireland, Galway*
*(3) Istituto di Oftalmologia, Università Cattolica, Rome*

**Purpose** Blood flow at the papilla depends upon the activity of the retina. This study investigated the reflectance changes of the optic disc (ch-R) induced by light stimulation and determined the origin and putative mechanisms underlying this effect.

**Methods** ch-R at 810 nm (isobestic wavelength) was measured from an area of the optic disc tissue (diameter 150 microns) during the variation of parameters pertaining to different stimuli (luminance, frequency and color ratio of diffuse chromatic flicker stimuli). Simultaneously with ch-R, the change in blood flow (ch-F) in the same area of the disc and the ERG 1F and 2F components were determined. Correlations were sought between ch-F, the 1F and 2F components and ch-R.

**Results** In response to the variation of the color ratio r of a 15-Hz chromatic flicker, ch-R and ERG 1F, as well as ch-R and ERG 2F, were significantly correlated. Significant correlations were also observed between ch-R and ch-F. Varying the frequency of a chromatic flicker with r = 0.8 (M-cone isolation) resulted in a significant correlation between ch-R and ERG 1F, but the association between ch-R and ch-F was not significant. ch-R and the ERG components did not correlate when the luminance of a 15-Hz flicker was varied, although ch-F and ERG 2F correlated significantly.

**Conclusion** Optic disc reflectance is coupled with retinal activity, the strength of this coupling depending upon the type of the retinal stimulation. ch-R is due in part to blood flow change and probably also to change in tissue scattering induced by increased axonal activity during light stimulation. Putative mechanisms underlying these ch-F and ch-R changes will be discussed in the light of current knowledge for the cerebral circulation.

## ▪ 3333
## Vasoactive Response of Porcine and Rabbit Ciliary Arteries to Unoprostone Isopropyl Ester and Free Acid Dissolved either in DMSO or Ethanol

*ALLEMANN RA*
*Laboratory of Ocular Pharmacology and Physiology, Basel*

**Purpose** To investigate vasoactive properties of unoprostone isopropyl ester and unoprostone free acid dissolved either in dimethylsulfate oxide (DMSO) or ethanol.

**Methods** Vasoactive changes (expressed in percent of 100 mM potassium chloride (KCl)-induced contraction) were assessed with a myograph system for isomteric forces measurements in quiescent, 100 mM KCl-, and/or 1 µM endothelin-1-precontracted vessels. Vessels were exposed to cumulative increasing concentrations (0.1 nM to 0.1 mM) of the drugs that were either dissolved in DMSO or ethanol. Level of significance was set at 5%.

**Results** In ethanol, unoprostone free acid induced in porcine ciliary arteries significant contractions in quiescent, KCl-, and endothelin-1-precontracted vessels (0.1 mM: 36.9% ± 7.6%, 21.7% ± 2%, 16.5% ± 19%, respectively). In contrast, in rabbit ciliary arteries, no significant contractions could be observed. In DMSO, unoprostone free acid induced in porcine ciliary arteries significant contractions only in KCl-precontracted vessels. No significant contractions could be observed in rabbit ciliary arteries. In ethanol or DMSO, unoprostone isopropyl ester induced both in porcine and rabbit KCl-precontracted ciliary arteries significant and marked relaxations (0.1 mM: 86.3% ± 13.1%, 79.5% ± 10.5%, respectively).

**Conclusion** Heterogeneity between porcine and rabbit ciliary arteries exist in the vasoconstrictive response to unoprostone free acid. The vasoconstricive response of unoprostone free acid was lower in presence of DMSO than of ethanol. Unoprostone propyl esther has vasodilating properties both in porcine and rabbit ciliary arteries.

## ▪ 3334
## Effect of Indomethacin on Optic Nerve Head CO2-Induced Dilation

*PETROPOULOS IK, MUNOZ JL, POURNARAS CJ*
*Department of Ophthalmology, Geneva University Hospitals, Geneva*

**Purpose** To search for mediators of CO2-induced dilation of the optic nerve head vessels. Prostaglandins and nitric oxide (NO) have been shown to mediate CO2-induced vasodilation in the retina.

**Methods** We measured the PO2 at intervascular areas of the optic disc in 17 anaesthetized mini-pigs using O2-sensitive microelectrodes placed at less than 50 µm from the optic disc. PO2 was measured continuously during 10 min under normoxia, hyperoxia (breathing of 100% O2), carbogen breathing (95% O2, 5% CO2), and hypercapnia (40% increase in inhaled CO2). Measurements were repeated after intravenous injection of the prostaglandin inhibitor indomethacin or the NO-synthase inhibitor L-NAME.

**Results** Before the injections, we observed a slight increase in optic disc PO2 during hypercapnia (ΔPO2=1.9±1.7 mmHg) or hyperoxia (ΔPO2=3.4±1.6 mmHg), but a much larger increase during carbogen breathing (ΔPO2=12.3±5.2 mmHg). After injection of indomethacin, the optic disc PO2 increase was similar during hyperoxia (ΔPO2=5.6±2.2 mmHg) or carbogen breathing (ΔPO2=5.8±3.2 mmHg). Moreover, the optic disc PO2 variation was minimal in hypercapnia after injection of either indomethacin (ΔPO2=0.5±1.9 mmHg) or L-NAME (ΔPO2=0.7±1.3 mmHg).

**Conclusion** Indomethacin fully inhibits the dilating effect of increased PaCO2 on the optic disc vessels, leading to a similar moderate increase in optic disc PO2 during carbogen breathing as in hyperoxia. In addition, indomethacin or L-NAME inhibits the optic disc PO2 increase seen during hypercapnia. Those results indicate that prostaglandins and, to a lesser extent, NO are mediators of CO2-induced dilation of the optic disc vessels. CO2-induced dilation of the optic nerve head vessels is probably due to a combined effect of prostaglandins and NO.

■ **3335**

## Indomethacin Reduces Optic Nerve Oxygen Tension

*STEFANSSON E (1), PEDERSEN DB (2), NORGAARD MH (2), EYSTEINSSON T (1, 3), KIILGAARD JF (2), LA COUR M (2), BANG K (4), JENSEN PK (2)*
*(1) Ophthalmology, University of Iceland, Reykjavik*
*(2) Ophthalmology, University of Copenhagen, Copenhagen*
*(3) Physiology, University of Iceland, Reykjavik*
*(4) MSD, Copenhagen*

**Purpose** CO2 breathing and carbonic anhydrase inhibition dilate blood vessels and increase oxygen tension in the central nervous system including retina and optic nerve. In order to study the mechanism of this effect and the role of cyclo-oxygenase and the prostacyclin pathway ,we investigated how indomethacin affects the optic nerve oxygen tension (ONPO2) in the pig and the ONPO2 rising effect of CO2 breathing and carbonic anhydrase inhibition.

**Methods** ONPO2 was measured in 5 pigs with a polarographic oxygen electrode placed 0.5 mm above the optic disc. 3% CO2 breathing was induced for 30 minutes; when ONPO2 had returned to baseline, 300 mg indomethacin was injected intravenously. 60 minutes hereafter, 3% CO2 breathing was induced for 30 minutes again, and when back to baseline 500 mg dorzolamide was injected. ONPO2, arterial blood pressure, heart rate and arterial blood gasses were recorded continuously.

**Results** Baseline ONPO2 (mean ± SD) was 2.33±2.4 kPa (n=5). Administration of 300 mg indomethacin decreased ONPO2 by –1.22±0.43 kPa (n=5). CO2 breathing increased ONPO2 significantly by 0.66±0.23 kPa and nonsignificantly by 0.17±0.25 when indomethacin had been given (n=4). 500 mg dorzolamide increased ONPO2 significantly by 0.22±0.12 kPa (n=5) after indomethacin. The dorzolamide effect in indomethacin treated pigs was significantly smaller than the effect previously found in untreated pigs.

**Conclusion** Systemic administration of indomethacin causes a decrease in ONPO2, probably due to decreased blood flow through vasoconstriction of vessels in the optic nerve and retina. Additionally, indomethacin blocks the ONPO2 increasing effect of CO2 breathing and carbonic anhydrase inhibition.

■ **3336**

## Effect of latanoprost on choroidal blood flow regulation in healthy humans

*SCHMETTERER L (1, 2), FUCHSJÄGER-MAYRL GF (1, 3), WEIGERT G (1), POLSKA E (1), KARL K (1)*
*(1) Clinical Pharmacology, Vienna*
*(2) Biomedical Engineering and Physics, Vienna*
*(3) Ophthalmology, Vienna*

**Purpose** Topical antiglaucoma drugs may exert ocular hemodynamic effects via two pathways. On the one hand pharmacologically-induced effects may occur at the posterior pole of the eye if the drug is accumulated at sufficiently high concentrations. On the other hand effects may be exerted because of the ocular hypotensive actions of these substances leading to an increase in ocular perfusion pressure. In the present study we investigated whether latanoprost may alter choroidal pressure-flow curves.

**Methods** 30 healthy male subjects were studied in a randomized, placebo controlled, double-masked two way cross-over design. Patients were randomized either to the placebo group (n = 15) or to the latanoprost group (n = 15) group. Before drug administration and after 14 days of topical medication choroidal blood flow regulation was assessed using laser Doppler flowmetry during an artificial increase in intraocular pressure using a suction cup and during isometric exercise. Choroidal pressure/flow relationships during these interventions were compared.

**Results** As expected latanoprost significantly reduced intraocular pressure ($p < 0.05$), but did not alter systemic blood pressure. Latanoprost did not affect baseline choroidal blood flow, but altered the choroidal blood flow response during artificial changes in ocular perfusion pressure ($p < 0.05$).

**Conclusion** These data indicate that choroidal blood flow regulation is modified by topical latanoprost. The results of the present study are well compatible with the idea that this effect is at least partially related to the IOP-lowering effect of the drug.

■ 3341

## Clinical features of ring melanoma of the uvea: A retrospective report on twenty-three patients

*AKGUEL H, ANASTASSIOU G, SCHILLING H, SCHUELER A, BORNFELD N*
*department of ophthlamology, University, Essen*

**Purpose** Ring melanoma of the uvea is a rare manifestation of uveal melanoma.It may present as masquerade syndrome mimicry pigmentary glaucoma and other conditions. To further improve our knowledge we present clinical and histological findings on twenty-three cases

**Methods** Retrospective analysis of 23 consecutive cases of ring melanoma treated in the department of ophthalmology at university of Essen over a 7,5-year period was performed.Patients with histologically proven findings to be ring melanoma were included in the series.Clinical features, management and histopathology were evaluated

**Results** Mean patient age was 61 years.Mean intraocular pressure at presentation was 36 mmHg.Blurred vision was found in 10 (43,5%) cases, pain in 6 (26,1%), both blurred vision and pain in 5 (21,7%) cases.Two patients (8,7%) were asymptomatic.21 (91,3%) patients had visible melanoma infiltration of anterior chamber angle,17 (80,9%) of 21 circumferential involvement.In 21 (91,3%) cases the iris was affected with mean 3,9 hours,in two (8,7%) cases iris was initially inconspicuous.Associated findings included in particular conjunctival injection,pupil deformation,corneal edema and prominent episcleral vessel.The tumor was managed with enucleation in all 23 (100%) cases.In 9 cases diagnosis was confirmed by biopsy prior to enucleation.In 2 cases enucleation followed after initial plaque radiotherapy for presumed ciliary-iris melanoma.Histopathological examination revealed spindle cell-type in 14 (63,6%) and mixed cell-type in 8 (36,4%) cases.In one case enucleation was performed elsewhere

**Conclusion** Early detection can prevent frustrane therapy because of misdiagnosis and reduce the potential risk of metastasis as a result of delay

■ 3342

## Prognostic factors of liver metastases from uveal melanoma

*KODJIKIAN L (1), RIVOIRE M (2), GARWEG JG (3), GRANGE JD (1)*
*(1) Croix-Rousse Hospital, Lyon*
*(2) Centre Léon Bérard, Lyon*
*(3) Inselspital, Bern*

**Purpose** This study was designed to assess survival and identify prognostic factors for liver metastases diagnosed by systematic screening in uveal melanoma patients

**Methods** Among 602 consecutive patients treated over 10 years for uveal melanoma and followed by systematic semi-annual hepatic screening (abdominal ultrasonography), 63 (10.5%) developed liver metastases; these patients form the basis of this study. Factors including patient demographics, characteristics of the uveal tumor, metastasis-free interval, severity of liver metastatic involvement, and treatments of metastases were studied retrospectively regarding their prognostic value, using univariate (Kaplan-Meier method) and multivariate (Cox model) analyses.

**Results** Thirty-five patients (55.6% of the metastatic population) received systemic chemotherapy or best supportive care only; 14 patients diagnosed with diffuse liver involvement had cytoreductive surgery and intra-arterial chemotherapy; 14 had complete surgical removal of liver metastases followed by postoperative intra-arterial chemotherapy. No significant surgical complications were experienced. The median overall survival after diagnosis of liver metastases was 15 months. It reached 25 months for selected patients with complete resection (P=0.0002). In this cohort of 63 patients, ten or fewer preoperatively diagnosed metastases and primary uveal melanoma not involving the ciliary body were independently associated with better prognosis.

**Conclusion** This study suggests that selected patients with screened liver metastases from uveal melanoma may benefit from aggressive treatment, including surgery. The two independent favorable prognostic factors are fewer than ten metastases at screening and the absence of ciliary body involvement.

■ 3343

## Age at diagnosis and bilateralization of unilateral retinoblastoma

*HADJISTILIANOU T (1), DE FRANCESCO S (1), LORE C (2), MASTRANGELO D (1), MENICACCI F (3)*
*(1) Ophthalmology - Ocular Oncology Unit, Siena*
*(2) Forensic Medicine, Siena*
*(3) Ophthalmology, Siena*

**Purpose** to show that the most important risk factor of metachronous tumor development in the fellow eye in unilateral retinoblastoma, is an age at diagnosis of less than 12 months, while both the number of tumor foci at diagnosis and family history play only a marginal role, if any.

**Methods** retrospective investigation on 167 unilateral retinoblastomas, diagnosed between 1980 and 2003.

**Results** 11 out of 167 patients (6.5%) developed tumors in the fellow eye after diagnosis of unilateral retinoblastoma. 12 out of 167 (7.1%) had a positive family history for the disease, but only three of them later developed tumors in the fellow eye. Multiple foci were detected in 23 out of 140 patients (27 cases not evaluated), but only one patient with multiple foci at diagnosis, later developed tumors in the other eye. Finally all cases (11) who developed metachronous bilateral retinoblastoma, had had a diagnosis of unilateral retinoblastoma before the age of 12 months, while none of those who were diagnosed after that age, later developed metachronous bilateral tumors.

**Conclusion** an age at diagnosis of less than 12 month seems to be the only relevant risk factor for metachronous tumor development in the fellow eye in unilateral retinoblastoma, but, strangely enough, a positive family history does not seem to influence this outcome

■ 3344

## Chemoreduction and local treatment for intraocular retinoblastoma

*ROMANOWSKA DIXON B (1), KOBYLARZ J (1), PIWOWARCZYK A (1), BALWIERZ W (2), DILUZNIEWSKA A (2)*
*(1) Dept of Ophthalmology of Jagiellonian University, Krakow*
*(2) Dept of Pediatric Oncology and Hematology, Krakow*

**Purpose** To evaluate the results of combined treatment for intraocular retinoblastoma.

**Methods** We examined children with unilateral and bilateral disease, treated in our Department from January 1996 to January 2005.

**Results** In I –III group according to R-E, it was possible to save more than 90% of eyes. In group IV-V we saved 20% of eyes.

**Conclusion** In I –III group according to R-E, it was possible to save more than 90% of eyes. In group IV-V we saved 20% of eyes.

## ▪ 3345
## Eyelid tumors: clinicopathological correlation of 1334 cases

*MENDEZ MC (1), SARASA JL (2), GARCíA-SANDOVAL B (3), BLANCO G (1), SAORNIL MA (1), FERNANDEZ N (1), PIGAZO JM (1)*
*(1) Registro de Patologia Ocular Miguel N. Burnier. Instituto Universitario de Oftalmobiología Aplicada (IOBA), Valladolid*
*(2) Departamento de Anatomía Patológica. Fundación Jiménez Díaz, Madrid*
*(3) Departamento de Oftalmología. Fundación Jiménez Díaz, Madrid*

**Purpose** To study epidemiological, clinical and pathological aspects in a series of eyelid tumors, and to compare benign and malignant tumors for those variables. To determine the clinical diagnostic sensitivity and specificity, and the pathological base of ocular cutaneous horns.

**Methods** 1334 eyelid tumors were processed and paraffin embedded, and light microscopic study was made. Epidemiological and clinical aspects (sex, age, localization and size of the lesions, clinical diagnosis…) were also collected. Statistical analysis was made, by comparison of percentages and Chi square test.

**Results** Benign tumors were 68%, being 57% of epithelial origin.The most frequent benign tumor was the seborrheic keratosis, and the most frequent malignant one was the basal cell carcinoma (the most frequent tumor in total). Benign tumors use to be located in the upper eyelid, while malignant tumors do so in lower eyelid and internal cantus. Size was bigger in malignant tumors. There is a statistical correlation between older ages and the development of malignant tumors (and of course between childhood and benign tumors).The pathological base of cutaneous horns was, most of all, verruca vulgaris, actinic keratosis and seborrheic keratosis. None of the cutaneous horns had a malignant base. Accuracy in the diagnosis showed a sensitivity of 83% and a specificity of 88%.

**Conclusion** Frequency of eyelid tumors received in our pathology laboratories has increased during the last ten years. There is a predominance of benign tumors and most of them are epithelial, followed in order by the adnexal and melanocytic ones. Sensitivity and specificity for clinical diagnosis is acceptable. * Partially funded by grant "Jose Maria Aguilar Bartolome"

## ▪ 3346
## Benign mixed tumour of the eyelid

*DIXON RS, SWEDO RJ*
*Bon Secours Hospital / Pathology, Grosse Pointe, Michigan*

**Purpose** BENIGN CUTANEOUS MIXED TUMOUR OF THE EYELID IS EXTREMELY RARE. WHEN 207 PERIOCULAR TUMOURS WERE REVIEWED ONE EXAMPLE WAS SEEN. AN OCULOPLASTIC INTERNET STUDY GROUP POLLED ITS MEMBERS. THEY REPORTED NINE ANECDOTAL CASES THAT WERE AT OR ABOVE THE MARGIN OF THE EYELID. ISOLATED CASE REORTS CAN BE FOUND IN THE LITERATURE. IT IS IMPORTANT TO DOCUMENT ANY ADDITIONAL CASE WHEN IT IS IDENTIFIED

**Methods** A CASE REPORT

**Results** A 50-YEAR OLD MAN PRESENTED WITH A THIRD-TIME RECURRENT TUMOUR OF THE MARGIN OF THE LOWER EYELID. THE TUMOUR WAS FIRM AND NODULAR. THERE WAS NO CLINICAL EVIDENCE OF MALIGANANCY. EXCISION AND REPAIR OF THE RESULTING DEFECT WERE PERFORMED. HISTOPATHOLOGICAL EVALUATION SHOWED A FAIRLY CIRCUMSCRIBED LESION WITH EPITHELIAL AND STROMAL COMPONENTS. THERE WAS APOCRINE DIFFERENTIATION. THE GLANDS WERE EMBEDDED WITHIN A MYXOID TO HYALINIZED STROMA.

**Conclusion** BENIGN MIXED TUMOUR OF THE EYELID IS A TUMOUR OF UNCERTAIN HISTOGENESIS. IT MUST BE CONSIDERED IN THE DIFFERENTIAL DIAGNOSIS OF OCULAR ADNEXAL TUMOURS. EXCISION WITH THE AID OF HISTOLOGICAL EXAMINATION OF MARGINS IS RECOMMENDED. PRIMARY REPAIR OF THE EYELID IS POSSIBLE EVEN FOR RECURRENT TUMOUR.

## ▪ 3347
## Metastasising Malignant Eccrine poroma masquerading as Basal Cell Carcinoma

*ARIF FA (1), WEIR R (2)*
*(1) Ophthalmology/ Stepping Hill Hospital, Stockport*
*(2) Instituite of ophthalmology, London*

**Purpose** The significance of multidisciplinary team work to manage Metastasising Malignant Eccrine poroma masquerading as Basal Cell Carcinoma.

**Methods** A 76 year old woman attending a routine 6 monthly glaucoma outpatient appointment was found to have a lower lid ulcerative lesion with rolled edges. A preliminary diagnosis of Basal cell carcinoma was made. A diagnostic biopsy was performed. Early staining of the slide suggested either a squamous cell carcinoma or a malignant eccrine poroma. Additional immuno-histochemical staining for epithelial membrane antigen confirmed the diagnosis of malignant eccrine poroma by confirming the presence of ductal differentiation. Extensive investigations did not show lymph node involvement. MRI scan reported a well defined soft tissue mass of the right upper cheek measuring 15mm x 20mm and not invading underlying facial musculature or involving regional lymph nodes. Surgical wide excision with rotational cheek flap was performed with the maxillofacial surgery department.

**Results** Uneventful recovery was recorded. 1 year review did not reveal any sign of recurrence. MRI assessment to define depth of tumour was important as a guide to excision method and extent. Follow up must be long term, bi-annual to annually as this tumour has a high recurrence rate requiring prompt re-excision.

**Conclusion** Malignant eccrineporoma metastasises much more frequently than BCC's around 20% versus 0.00027%. It may resemble a Basal cell carcinoma but requires wide excision. Malignant types metastasise via lymphatic and haematogenous spread in 20% of cases. Lymph node involvement has a poor prognosis with 65% mortality at 5 years when regional lymph nodes are involved and a similar recurrence rate at 5 years.

■ **3351**

## LASEK vs LASIK to correct high myopia

*TEUS MA (1, 2, 3), SáNCHEZ JM (1), GIL R (1, 4), ARRANZ M (1, 3), ORTEGA M (1)*
*(1) Vissum Hospital Oftalmológico de Madrid, Madrid*
*(2) Universidad de Alcalá, Alcalá de Henares*
*(3) Hospital Príncipe de Asturias, Alcalá de Henares*
*(4) Universidad Complutense de Madrid, Madrid*

**Purpose** To compare the refractive results of LASEK and LASIK performed to correct high myopia

**Methods** This is a prospective, observer masked study. We studied patients with myopia higher than –6 D in whom either LASEK or LASIK was performed. Both groups were refractive error matched. Refractive analysis was performed by a masked observer before and 1 day, 1 week, 1 month and 3 month postop. Mitomycin C was applied intraoperatively for one min. in every LASEK treated eye.

**Results** 39 eyes fulfilled the inclusion criteria in each group, and were included in the study. There were no differences either in the mean preop refractive error (-8.4 + 1.9D) , BCVA, age or gender between groups. We found the UCVA to be significantly better in the LASIK group than in the LASEK group in post op visits at day 1 and 7(p=0.001). In both the 1 and 3 month visits, there was no significant difference between groups. The residual refractive error was similar in both groups (p=0.3).

**Conclusion** Visual improvement after LASEK (with adjunctive mitomycin C) is significantly slower than after LASIK to correct high myopia. However, no significant difference was found after one month the refractive result of both techniques.

■ **3352**

## Comparative study of silicone and non silicone hydrogel soft contact lenses used as bandage after LASEK

*GIL R (1, 2), ARRANZ E (1, 3), HERNANDEZ-VERDEJO JL (1, 2), FUENTES I (1), TEUS MA (1, 3, 4)*
*(1) Vissum Hospital Oftalmológico de Madrid, Madrid*
*(2) Universidad Complutense de MAdrid, Madrid*
*(3) Hospital Príncipe de Asturias, Alcalá de Henares*
*(4) Univesidad de Alcalá, Alcalá de Henares*

**Purpose** To evaluate two different soft contact lens materials as a continous-wear bandage contact lens after LASEK surgery.

**Methods** This is a prospective, single masked, interventional study. The inclusion criteria were myopia lower than -6.00D, astigmatism lower than -1,5D; BCVA $^{3}$20/20, in both eyes.Patients were ramdomized fitted with balafilcon A (Pure Vision) in one eye and Poly-2-Hydroxietilmetacrilate-co-metacrilate glycerol (Equis 60) in the other. High contrast visual acuity, corneal epithelium status, conjuctiva hyperemia, lens movement, contact lens debris and subjective comfort questionaire were assessed. Patients wore continuosly both contact lenses for 5 days. Measures were taken before and at 1 day and 5 days after the procedure. The ANOVA and paired two tails Student´s T test analyses were performed.

**Results** We analyzed 32 eyes of 16 consecutive patients who underwent LASEK for low to moderate myopia. The mean equivalent spherical was – 3,25D.There was no difference in high contrast visual acuity, conjuctiva hyperemia, lens movement, contact lens debris and subjective comfort between lenses at any visit. In contrast, corneal epithelium status was statistically better in the silicone contact lens eye at 5 days after LASEK (p: 0,01).

**Conclusion** Our results suggest that the type of contact lens used after LASEK procedure has an influence in the corneal epithelium healing.

■ **3353**

## Changes in retinal nerve fiber layer thickness induced by LASIK surgery

*ARRANZ E (1, 2), ROMAN JM (1), MARINA C (1), ALVAREZ M (1), TEUS MA (1, 2, 3)*
*(1) Vissum Hospital Oftalmológico de Madrid, Madrid*
*(2) Hospital Universitario Príncipe de Asturias, Alcalá de Henares*
*(3) Universidad de Alcalá, Alcalá de Henares*

**Purpose** To evaluate changes occurred with LASIK in retinal nerve fiber layer (RNFL) thickness.

**Methods** This is a pilot study. We have selected 20 eyes of ten patients who had myopic LASIK and were operated with M2 microkeratome carrying the instructions in the selection of rings, stoppers, and heads attending the keratometry values of the eyes.We have done OCT preop, one and three months postop.

**Results** 20 LASIK operated eyes has been evaluated.Mean age was: 33.29 +- 6.65 years, and the mean spherical equivalent were: -3.113 +- 1.25 dioptries. The mean intraoperative suction time was: 18 +-3.5 seconds.The parameters evaluated were: RFNL average, RFNL inferior, RFNL superior, RFNL nasal and RFNL temporal and finally retinal thickness average (foveal thickness). We have a statistical significant difference in RFNL average: 101.02 +-9.7 microns preop vs 97.686 +-9.335 microns at one month (p=0.001) and 97.66 +- 9.782 microns at three month (p=0.001) and in RNFL inferior: 128.433 +- 17.651 microns preop vs 125.12 +- 15.742 microns at three month (p=0.03), and in RNFL superior: 118.457 +-18.785 microns preop vs 113.237 +- 20.301microns at one month (p=0.02), and in RNFL nasal: 74.186 +-18.014 microns preop vs 69.66 +-19.128 microns at three months (p=0.02), and in RNFL temporal: 77.643 +- 16.8 microns pre vs 74.873 +- 18.295 microns at one month and 74.01 +-15.738 microns at three month (p=0.002), and in retinal thickness average: 175.55 +- 28.797 microns preop vs 169.644 +- 26.637 microns at one month (p=0.002) and 171.06 +- 25.395 microns at three months (p=0.02).

**Conclusion** These results suggest that the increase of IOP during LASIK could affect to RFNL and foveal thickness.

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## The Nature and Fate of Tear Lipids in Contact Lens Wear

*MANN AM, CAMPBELL D, GRIFFITH G, TIGHE B*
*Biomaterials Research Unit, Birmingham*

**Purpose** The role & structure of the layers of the tear film is a subject of ongoing discussion in ophthalmology and the increased use of silicone hydrogels has produced renewed interest in the interaction of lipids with contact lenses. In particular the polar lipids & their ability to act as an aqueous-lipid interface are of importance. Oxidation of lipids leads to their degradation and dysfunction. This poster shows techniques that are being developed to probe the role of polar tear lipids & to study the interaction of the tear film lipid layer with (silicone hydrogel) contact lenses

**Methods** Chromatographic, fluorescence & mass spectrometric are used for compositional analysis of the lipid layer of tear film. Spinning drop tensiometry is used to study the interfacial chemistry of the individual lipids and the tear film. Tear lipid degradation products are analysed using MDA and FOX analysis. The depth of lipid penetration into lenses is investigated using novel techniques (PEEMS & PEEFS). The effects of introducing lenses into the tear film will also be investigated using these techniques. Lenses & tear samples are obtained from a variety of clinically managed programmes

**Results** Irrespective of composition or surface coating technique lipid accumulation occurs rapidly on all commercially available silicone hydrogel lenses. Spinning drop tensiometer studies show that the polar phospholipids are essential to enable the rapid spreading of the lipid layer over the aqueous.

**Conclusion** While broad similarities exist in lipid composition between subjects the analyses of the phospholipids and lipid oxidation products are of interest to establish their correlation with patient-related wear problems

■ 3355 / 236

## Visual recovery following PRK using corneal epithelium brushing and alcoholic dehydration : a confocal microscopy study

*PICCIRILLO V, DI NUZZO A, RAO V*
*Ospedale Civile di Maddaloni ( ASL CE / 1 ), Maddaloni*

**Purpose** To study visual recovery and confocal microscopy findings three months after PRK using mechanical (brushing) and chemical (alcoholic) corneal epithelium removal .

**Methods** 120 corneas of 60 patients (30 males,30 females,average:36.4 y. o) were examined by slit-scanning confocal microscope (CONFOSCAN 2) 3 months after correction of simple myopia (average - 5.50 D. ) using PRK technique ( Laser Schwind ) . Male and female patients were at random divided into two groups :group 1,who received corneal epithelium brushing, group 2,who received epithelium dehydration.Patients were matched for age,sex and ethnicity.Visual recovery (Snellen chart)and Confoscan findings were related in the short term follow up (3 months) .

**Results** 3 months after PRK mean visual acuity was not different between the groups. Confocal microscopy did not reveal morphological differences within the corneal epithelium and anterior part of stroma between the groups :irregular pattern of elongated keratocytic nuclei were found in 50 % of both groups patients.The only difference was the rapidity of corneal riepithelization after PRK as evaluated by slit lamp:a complete corneal riepithelization was observed in the forth day in 90% of group 1 patients,in the fifth day in 60% of group 2 patients .

**Conclusion** Mechanical and chemical corneal epithelium removal does not influence visual recovery after PRK .Confoscan examination ( CONFOSCAN 2 ) does not reveal morphological differences between the techniques within the corneal epithelium and anterior part of stroma . The rapidity of complete corneal riepithelization is higher in patients treated with corneal brushing as revealed by slit lamp examination .

■ 3356 / 237

## Influence of multipurpose solutions on the protein composition of the tear film

*GRUS FH, BOZKURT N, PFEIFFER N*
*Dept. of Ophthalmology, University of Mainz, Mainz*

**Purpose** To analyze the tear protein profiles of non-contact lens wearers, and of contact lens wearers using different multipurpose solutions (MPS) for cleaning and storage.

**Methods** Wearers of soft contact lenses were recruited and allocated to use either Optifree Express MPS or Complete MPS for 4 weeks (n = 20 in each group). Tears were collected and analyzed before starting use of solutions, and at 1, 2, and 4 weeks after starting use. Tears were also collected and analyzed from 20 control patients (non-contact lens wearers), who were not exposed to either MPS. Multivariate statistical analysis of protein profiles was used to determine the normality of tear protein composition. Specific protein biomarkers were found by means of ProteinChips (SELDI-TOF) with subsequent multivariate statistics and artificial neural networks, and identified using tandem mass spectrometry (LC-MSMS).

**Results** Before starting use of solutions, tear protein composition in all contact lens wearers deviated from tear composition in normal controls (non-contact lens wearers). After 4 weeks using the different care regimens, tear protein composition of patients using OptiFree Express MPS was further deviated from normal. In contrast, tear protein composition of patients using Complete MPS returned towards normal. In fact, the tear composition of over 50% of Complete MPS users was classified as "normal" rather than "contact lens wearer" at 4 weeks. Using MPS, a decrease of inflammatory markers and an increase of potentially protective markers could be clearly demonstrated.

**Conclusion** Contact lens wear alters tear protein profiles in a complex manner. The use of MPA solutions such as Complete returns the tear profile towards normal.

■ 3357 / 238

## Frictional and lubricity changes in contact lenses during wear

*MAHOMED A, NASSO M, TIGHE BJ*
*Biomaterials Research Group, Aston University, Birmingham*

**Purpose** Friction and lubricity are important aspects of the interaction between contact lenses and the anterior eye and the in vivo behaviour of ophthalmic solutions. Although instrumentation is available for the measurement of artificial joint components, there are as yet no established in vitro techniques that reflect the sliding motion between the eyelid and the anterior surface of the lens during blinking. We have investigated technique development in this area in order to measure the coefficient of friction (μ) of contact lenses before and after wear.

**Methods** A high sensitivity tribometer has been identified and adapted for the study of contact lenses. The lens is placed on a convex mould which slides against a moving substrate (which can be varied) in the optional presence of an appropriate lubricating solution (tears or artificial tear solution). The resistance to motion is expressed in terms of μ.

**Results** Using this instrument μ values down to 0.001 can be measured reproducibly. Lenses from a series of clinically managed wearer trials have been studied. The technique shows clear material-dependant differences in μ values of unworn lenses (0.005–0.1). More interestingly are changes in the frictional behaviour of lenses as a consequence of wear (0.05-0.2).

**Conclusion** Three distinct contributions can be discerned. The first is due to progressive front surface dehydration during wear, observed only if lenses are examined directly on removal from the eye. The second is due to deposition and subsequent degradation of tear components, principally lipid and protein. The third is due to changes in the surface chemistry of the lens material, typified by, and most marked in, the loss of polyvinyl alcohol during wear from Focus® Dailies® lenses.

■ 3358 / 239

## Novel Ophthalmic Biomaterials

*MAHOMED A, HENLEY C, TIGHE BJ*
*Biomaterials Research Unit, Aston University, Birmingham*

**Purpose** The synthesis of new hydrogels with suitable properties to enable them to be used as biomimetic synthetic cornea, corneal inlays & onlays, & "third generation" contact lens materials.

**Methods** The human cornea provides precise targets in terms of mechanical & surface properties. Due to the cornea having a complex & composite structure it is not possible to mimic its properties with homogenous hydrogels. The approach used here is to synthesise Semi Interpenetrating Polymer Networks, which mimic to some extent the Interpenetrating Polymer Network of natural structures such as cornea & intervertebral disc. To achieve this it has been necessary to extend the range of monomers conventionally used in ophthalmic biomaterials in order to obtain a wider range of solvation & partitioning properties of the constituent monomers.

**Results** A group of N-vinylamides used singly or in combination has allowed the target structure to be achieved. These monomers have also enabled the synthesis of a range of novel copolymers, which are particularly useful in the synthesis of contact lens material that combines flexibility with high oxygen permeability. A hydrophilicity series of these amides has been determined by measuring the Equilibrium Water Contents of copolymers consisting of 70%(w/w) 2-hydroxyethylmethacrylate and 30%(w/w) N-vinylamide. The following series was obtained: N-vinylacetamide (63%) > N-methyl-N-vinylacetamide (NMNVA) (53%) > N-vinylpyrolidone (48%). In addition NMNVA has been found to have excellent solvation properties, is compatible with a variety of conventional monomers, & has low cytotoxicity.

**Conclusion** N-vinylamides are a group of monomers that can potentially revolutionise ophthalmic biomaterials in terms of their excellent solvation, partitioning & compatibility properties.

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## Silicone Hydrogels: Trends in Products and Properties

*FRANKLIN V, ROSS G, NASSO M, LYDON F, TIGHE B*
*Biomaterials Research Unit, Birmingham*

**Purpose** Although silicone hydrogels resemble conventional hydrogels because of the water that they contain, the substantial presence of relatively hydrophobic silicone components leads to many differences in behaviour from that of simple mid to high water content hydrogel lenses. In the six years since their launch, clinical experience has revealed a combination of characteristic benefits and complications (such as mucin balls and SEALS).

**Methods** This poster compares the dynamic mechanical properties, dynamic wettability, and frictional properties of galyficon A, lotrafilcon-A and balafilcon-A (together with such other materials as become available for characterisation) in comparison to two reference points. The first of these is typical conventional hydrogel behaviour in the mid-water content range. The second is the human cornea.

**Results** Taken together these properties provide a basis for interpreting the clinical behaviour of silicone hydrogels in comparison with conventional soft lens materials.

**Conclusion** The relative behaviour of materials will be summarised on the poster and the fact that current silicone hydrogels are significantly different fromconventional hydrogels and also from each other highlights the need for standardised relevant in-vitro methods of assessment for these materials

■ 3361

## Mechanism of polyol pathway-induced acute and slow-developing diabetic cataracts

*CHUNG SSM, CHAN AWH, CHUNG SK*
*Institute of Molecular Biology, University of Hong Kong, Hong Kong*

**Purpose** To determine the mechanism of slow-developing diabetic cataract

**Methods** Examine the rate of cataract development by slitlamp microscope in the following lines of mice: (1) Trangenice mice overexpressing aldose reductase in their lenses (ARtg), (2) Sorbitol dehydrogenase deficient (SDH-/-) mice, (3) ARtg/SDH-/- double mutant mice, (4) glutathione peroxidase-1 deficient (GPX-1) mice, (5) ARtg/GPX-1 double mutant mice. All the mice were non-diabetic. Some mice received vitamin E treatment to reduce oxidative stress.

**Results** The ARtg and SDH-/- mice started to develop cataract about 1 year after birth because of high accumulation of sorbitol in their lenses. The ARtg/SDH-/- double mutant mice developed cataract beginning at around 3 months old, indicating that increased sorbitol accumulation hastened cataract development. Vitamin E treatment significantly delayed cataract development in these mice, indicating that oxidative stress also contribute to the development of these cataracts. This was confirmed by the fact that ARtg/GPX-1 double mutant mice developed cataract much faster than the ARtg mice.

**Conclusion** The non-diabetic ARtg and SDH-/- mice simulate the moderately hyperglycemic patients in that they accumulate moderate amounts of sorbitol in their lenses. They develop cataract spontaneously after about 1 year old. In these slowing-developing diabetic cataract models, polyol pathway-induced osmotic stress and oxidative stress both contribute to cataract development.

■ 3362

## Glycemic and Oxidative stress in the Lens. Implications on Cataract Formation in diabetes

*HEGDE KR (1), VARMA SD (1, 2)*
*(1) Ophthalmology, Baltimore*
*(2) Biochemistry, Baltimore*

**Purpose** One of the central biochemical manifestations of diabetes is the inhibition of cellular metabolism apparent by the fall in respiratory quotient, diverting inspired oxygen from the cytochrome linked repiratory pathway to the auto-oxidative pathway generating oxyradicals and consequent oxidative stress. The purpose of the present study was to examine the superimposition of this stress over the age-related oxidative stress existing independent of diabetes.

**Methods** Diabetes was induced in S. D. rats by I.P. streptozotocin. Lenses isolated from the diabetic and normal (control) animals were cultured in medium 199 and their ability to accumulate 86Rb+ against the electrochemical gradient was measured as an index of the lens membrane transport function. ATP and GSH levels, serving as indices of metabolic and antioxidant status of the tissue, were also measured. In parallel experiments, these biochemical parameters were determined in lenses incubated in the presence of menadione (redox cycler generating superoxide and other ROS).

**Results** The uptake of rubidium was depressed in the diabetic lenses, along with the decrease in ATP and GSH. Similar decreases were observed in the normal lenses when exposed to menadione. The depression in the case of diabetic lenses was further enhanced in the presence of menadione.

**Conclusion** In view of the enhancement of the physiological and biochemical damage to the diabetic lenses in presence of menadione, as compared to the normal lenses, we conclude that the diabetic lenses are excessively susceptible to oxidative stress. Accelerated cataractogenesis in diabetes hence appears to be due to a combination of the metabolic inhibition and the age associated increase in ambient ROS generation.

■ 3363

## Genetic Studies of Aldo-Keto Reductases and Diabetic Eye Disease

*PETRASH M, CHANG Q*
*Ophthalmology & Visual Sciences, Saint Louis*

**Purpose** The aldo-keto reductases (AKRs) comprise a superfamily of enzymes that catalyze the NADPH-dependent reduction of a variety of carbonyl compounds. Several members of this enzyme family are associated with eye diseases, including cataract, retinopathy, and glaucoma. The purpose of our studies is to develop genetic approaches to better understand physiological roles for individual AKRs.

**Methods** AKRs closely related in amino acid sequence to human aldose reductase were identified by BLAST algorithm queries of human, mouse, and yeast genomes. Corresponding genes were cloned into procaryotic expression plasmids, from which recombinant proteins were expressed and purified for kinetic and structural characterization. Open reading frames in yeast AKR genes were disrupted to produce strains that were functionally null for one or more AKRs.

**Results** At least five aldose reductase-like AKRs are expressed in yeast. Each demonstrates robust NADPH-dependent carbonyl reductase activity with a variety of carbonyl-containing substrates. Null strains are viable, but pronounced stress sensitivity is observed when three or more AKR open reading frames are disrupted. Rescue of such stress sensitivity phenotypes is achieved by transfection with catalytically active human aldose reductase. Gene array experiments demonstrated that AKR gene deletion substantially altered the stress-response transcriptome. Like yeast, mammalian genomes contain multiple aldose reductase-like genes. Overlapping kinetic properties were observed among three aldo-keto reductases in mouse. However, divergent physiological roles are evident based on differences in tissue distribution and gene regulation.

**Conclusion** Genetic studies will be necessary to understand the functional significance of the AKR gene family.

■ 3364

## Non-structural functions of crystallin

*BHAT S*
*Los Angeles*

**ABSTRACT NOT PROVIDED**

■ 3365

**Loss of beta-crystallin chaperone activity, a probable cause for diabetic cataract**

*ABRAHAM EC*
*Little Rock*

ABSTRACT NOT PROVIDED

■ 3421

## 24-hour IOP control with Travoprost

*KONSTAS AGP*
*Thessaloniki*

**Purpose** To evaluate the quality of 24-hour intraocular pressure (IOP) control between morning and evening dosed travoprost in POAG patients.Design: Prospective, crossover, double-masked comparison.

**Methods** Following a 6 week medicine free period, 33 POAG patients were randomized to receive travoprost dosed in the morning or evening. Following 8 weeks of treatment a 24-hour IOP curve was performed at 06:00, 10:00, 14:00, 18:00, 22:00 and 02:00. Patients were then treated with the opposite dosing regimen for another 8 weeks, after which the 24-hour IOP curve was repeated.

**Results** The untreated mean 24-hour IOP was 23.6 ± 2.0 mm Hg. There were no differences for mean 24-hour IOP between the morning (17.5 ± 1.9 mm Hg) and evening dosing (17.3 ± 1.9 mm Hg, P = 0.7). At 10:00 the evening dosing provided a statistically lower IOP (17.2 ± 2.1 mm Hg) compared to morning (19.1 ± 2.5 mm Hg, P = 0.02). There was a trend to lower IOP at 06:00 and 14:00 with evening dosing (0.6-0.9 mm Hg, P ≥ 0.1) and at 22:00 and 02:00 with morning dosing (0.9-1.0 mm Hg, P = 0.1). Furthermore, evening dosing demonstrated a statistically lower 24-hour fluctuation of IOP (3.2 ±1.0 mm Hg) compared to morning dosing (4.0 ± 1.5 mm Hg, P = 0.01). Safety was similar between groups.

**Conclusion** This study suggests that both morning and evening dosing of travoprost provide effective 24-hour IOP reduction. However, the evening dosing of travoprost demonstrates greater daytime efficacy with a significantly narrower fluctuation of 24-hour pressure.

■ 3422

## Efficacy and safety of carbonic anhydrase inhibitors as adjunctive therapy to a PGA

*CARASSA RG*
*University of Milan, Milan*

**Purpose** To provide an overview of topical carbonic anhydrase inhibitors (TCAIs) as adjunctive therapy to a prostaglandin analogue (PGA) for patients with primary open-angle glaucoma or ocular hypertension.

**Methods** A survey was conducted of recent published literature on TCAIs as adjunctive therapy to a PGA.

**Results** One study demonstrated a 41% decrease from baseline with the combination of a TCAI and a PGA. Other reports have shown additional reductions in IOP of up to a 5.3 mmHg (23.5%) when a TCAI is added to prostaglandin analogue monotherapy. Another recent clinical trial reported greater IOP lowering at months 1, 2 and 3 with concomitant travoprost and brinzolamide than with the fixed combination of latanoprost and timolol. Side effects reported from the concomitant administration of a TCAI and a PGA have been mild in nature.

**Conclusion** A topical carbonic anhydrase inhibitors appears to be a safe and effective adjunct to prostaglandin analogue therapy for those patients who require additional IOP lowering.

■ 3423

## Concomitant use of Travatan and Azopt vs. the fixed Latanoprost/Timolol combination

*GARCIA-FEIJOO J*
*Insitituto de Investigaciones Oftalmológicas Ramón Castroviejo, Hospital Clínico San Carlos, Universidad Complutense de Madrid, Madrid*

**Purpose** To compare the efficacy and safety of a regimen of travoprost 0.004% and brinzolamide 1.0% with a regimen containing the fixed combination of latanoprost 0.005%/timolol 0.5%.

**Methods** Forty-four patients with primary open-angle glaucoma or ocular hypertension were randomly assigned to receive concomitant administration of travoprost 0.004% once daily and brinzolamide 1.0% (TB, n = 22) twice daily with those of a fixed combination of latanoprost 0.005%/timolol 0.5% (LT, n = 22) once a day. The study consists of visits at screening, baseline and after 2 weeks, 1 month, 2 months and 3 months of therapy. IOP was measured at 9 a.m., 12 p.m. and 4 p.m. at each study visit. Adverse events were also recorded at each study visit.

**Results** Overall mean IOP was significantly lower in the TB compared to the LT group after 1 month, 2 month and 3 month follow-up. The 9 a.m. measurements were significantly different, reaching a maximum difference (16.9 ± 0.9 mmHg for TB vs 18.4 ± 1.8 mmHg for LT, P < 0.001) at the 3 month visit. The percentage of responders with IOP reductions ≥ 30% was higher in the TB group. Both treatments were safe and well tolerated.

**Conclusion** Travoprost 0.004% and brinzolamide 1.0% concomitant therapy demonstrated a greater IOP lowering efficacy and a greater percentage of responders than the fixed latanoprost 0.005%/timolol 0.5% combination.

■ 3424

## Azopt in adjunct therapy with Travatan vs Travatan monotherapy

*FRANKS W*
*Moorfields Eye Hospital, London*

**Purpose** The purpose of this study was to demonstrate that combined travoprost 0.004% and brinzolamide 1.0% therapy is superior in lowering intraocular pressure (IOP) compared to travoprost 0.004% alone in patients with open angle glaucoma or ocular hypertension.

**Methods** This was a single-arm, open-label study where the patients acted as theirown controls. Patients entered the study must have completed at least 6 weeks travoprost 0.004% (once daily) monotherapy. Brinzolamide 1.0% (twice daily) was subsequently added to their treatment regimen for the 12 week duration of the study. IOP was measured at baseline and following 4 and 12 weeks of travoprost 0.004% and brinzolamide 1.0% treatment. A total of 82 patients were enrolled, all were eligible for the safety while 79 of these patients were analyzed in the intent-to-treat population.

**Results** IOP was reduced (compared with the baseline on travoprost 0.004%) after 4 and 12 weeks of combined travoprost 0.004% and brinzolamide 1.0% therapy by an average of 3.9 mmHg (17.4%, P < 0.0001) and 4.2 mm Hg (18.4%, P < 0.0001), respectively. A greater percentage (up to 60.6%) of patients on combination therapy had an IOP lower than 18 mmHg. The most frequent adverse event was ocular hyperemia.

**Conclusion** Combined travoprost 0.004% and brinzolamide 1.0% therapy significantly lowers IOP compared to travoprost 0.004% monotherapy. The combination therapy resulted in a significantly greater percentage of patients achieving IOPs of less than 18 mmHg.

■ **3425**

## Clinical utility and update of fixed combinations

*DENIS Ph*
*Service d'ophtalmologie (Pavillon C), Hopital Edouard Herriot, Lyon*

**Purpose** To provide an overview of fixed combination glaucoma therapies.

**Methods** Literature survey of recently published clinical studies with fixed combination glaucoma agents.

**Results** Recent reports have discussed a carbonic anhydrase inhibitor/timolol (dorzolamide/timolol) and prostaglandin analog/timolol combination (travoprost/timolol or latanoprost/timolol). All of these therapeutic regimens have been shown to provide greater IOP lowering that either respective agent dosed alone. For example, recent studies with travoprost/timolol have reported IOP reductions from baseline of 8 to 10 mmHg with similar efficacy whether the combination drop was dosed once in the morning or once in the evening. The relationship of fixed combination therapies to factors that influence compliance such as side effects, cost, and complexity of the dosing regimen will be discussed.

**Conclusion** Fixed combination therapies may provide patient benefits such as enhanced IOP lowering efficacy compared with monotherapies, better compliance and reduced cost compared with concomitant therapeutic regimens.

■ 3431

## Involvement of active gelatinase B/matrix metalloproteinase-9 in vitreous hemorrhagic transformation of proliferative diabetic retinopathy

*ABU EL ASRAR AM (1), DESCAMPS FJ (2), MARTENS E (2), KANGAVE D (3), STRUYF S (4), GEBOES K (5), OPDENAKKER G (2)*
*(1) Dept. of Ophthalmology, College of Medicine, King Saud University, Riyadh*
*(2) Laboratory of Immunobiology, Rega Institute, Univ. of Leuven, Leuven*
*(3) Research Center, College of Medicine, King Saud Univesity, Riyadh*
*(4) Laboratory of Molecular Immunology, Rega Institute, Univ. of Leuven, Leuven*
*(5) Dept. of Morphology and Molecular Pathology, Univ. of Leuven, Leuven*

**Purpose** To investigate the presence and activation status of matrix metalloproteinase (MMP)-2 and MMP-9 in vitreous samples from patients with proliferative diabetic retinopathy (PDR).

**Methods** Vitreous samples were obtained from 151 consecutive patients undergoing vitrectomy for the treatment of retinal detachment complicated by proliferative vitreoretinopathy (PVR) (19 specimens), rhegmatogenous retinal detachment without PVR (RD) (65 specimens), and PDR (67 specimens). PDR patients were graded at the time of vitrectomy for the presence of hemorrhage and presence or absence of patent new vessels. Samples were assayed for total protein content, MMP activities by semi-quantitative zymography, and hemoglobin concentrations.

**Results** Total protein and MMP-2 levels in the vitreous samples of patients with PVR were significantly higher than the levels found in patients with PDR and in patients with RD ($p < 0.001$; $p = 0.0182$, respectively) and both correlated in the entire study group ($r = 0.5438$; $p < 0.001$). Pro-MMP-9 and active MMP-9 levels in the vitreous samples of patients with PDR were significantly higher than the levels found in patients with PVR and in patients with RD ($p<0.001$ for both comparisons). Active MMP-9 levels in the vitreous samples of PDR patients with hemorrhage (75.7 ⊠ 106.3 scanning units) were significantly higher than that in PDR patients without hemorrhage (7.1 ⊠ 16.2 scanning units) ($p < 0.001$). Active MMP-9 levels strongly correlated with hemoglobin levels in PDR patients ($r = 0.7525$; $p<0.001$).

**Conclusion** Active MMP-9 may play an important role in hemorrhagic transformation in patients with PDR.

■ 3432

## Immunoreactive ET-1 in the vitreous humour and epiretinal membranes of patients with proliferative diabetic retinopathy

*ROLDAN-PALLARES M (1), ROLLIN R (2), MARTINEZ-MONTERO JC (3), FERNANDEZ-CRUZ A (4), BRAVO-LLATA C (5), FERNANDEZ-DURANGO R (6)*
*(1) Ophthalmology. Hospital Clinico San Carlos. UCM, Madrid*
*(2) Research Uni .Hospital Clinico San Carlos, Madrid*
*(3) Ophthalmic Pathology. Hospital Gregorio Marañon, Madrid*
*(4) Medicine III.Hospital Clinico San Carlos. UCM, Madrid*
*(5) Mathematics.Complutense University (UCM), Madrid*
*(6) Research Unit. MedicineIII. Hospital clinico San Carlos, Madrid*

**Purpose** Endothelin-1 (ET-1), a potent vasoconstrictor with mitogenic properties, is a hyperglycaemia and hypoxia indicible factor. ET-1 gene expression is increased in diabetic rat retina. We investigate the potential role of ET-1 in the pathogenesis of proliferative diabetic retinopathy (PDR)

**Methods** Plasma and vitreous samples were collected from normal (n=25) and diabetic patients, these last with proliferative diabetic retinopathy (n=25) and with nonproliferative diabetic retinopathy (n=25). They must have epiretinal membranes (ERMs) or other ocular conditions candidates of vitrectomy. Immunoreactive endothelin-1 (IR-ET-1) was tested in plasma and vitreous by radioimmunoassay. Immunoreactive ET-1 was localized in ERMs immunohistochemically.

**Results** IR-ET-1 levels in plasma and vitreous were higher ($p<0,0001$) in diabetics than in the control group. These levels also were higher ($p<0,0001$) in patients with PDR than in those with nonproliferative diabetic retinopathy. Eyes with ERMs of the PDR group (PERMs) showed the highest IR-ET-1 vitreous levels (14,67 pg/ml). Immunoreactive-ET-1 was localized in the cellular and stromal components of ERMs of diabetic and non diabetic patients

**Conclusion** IR-ET-1 level in human vitreous is elevated in diabetic patients with PDR. Furthermore, immunoreactive ET-1 was localized in the cellular and stromal components of ERMs of diabetic and non diabetic patients. These data suggest that ET-1 is involved in retinal disease

■ 3433

## Cytokine gene polymorphism in retinal detachment patients with and without proliferative vitreoretinopathy. Preliminary study

*PASTOR JC (1), SANABRIA MR (1), GARROTE JA (1), TELLERIA JJ (2), GARCIA MT (1)*
*(1) Instituto de Oftalmobiologia Aplicada (IOBA), Valladolid*
*(2) Instituto de Biologia y Genética Molecular (IBGM), Valladolid*

**Purpose** Cytokines and others growth factors play an important role in the pathogenesis of proliferative vitreoretinopathy (PVR). Interindividual variations in the capacity of cytokine production has been shown to correlate with some single nucleotid polymorphisms (SNPs) in cytokine genes. The purpose of this study was to analyze the possible role of these SNP in the development of PVR.

**Methods** SNPs were analyzed in five cytokines (tumour necrosis factor-α (TNF-α), transforming growth factor β1 (TGF-β1), interferon γ (IFN-γ), interleukin 6 (IL-6) and interleukin 10 (IL-10)) in two groups of patients surgically treated of rhegmatogenous retinal detachment (RD): group RD (27 patients without PVR), and group PVR (31 patients with PVR) and an ethnically matched healthy control group. All individuals were genotyped as high or low producers of TNF-α and IL-6 and high, intermediate and low producers of TGF-β1, IFN-γ, and IL-10.

**Results** An elevated frequency of high producer phenotypes of TGF-β1 was found in PVR patients. The genotype distribution of the codon 10 polymorphism was different between PVR and RD patients (p=0,018) and between PVR and controls in codon 25 (p=0,011) There was a higher frecuency of allele T in codón 10 in PVR patients when compared with RD group. No statistically significant differences were observed between groups of patients and control group in the rest of SNPs

**Conclusion** An association between TGF-β1 genetic profile and the development of PVR has been detected in this study.Further studies are necessary to confirm this finding and to establish its clinical relevance.

■ 3434

## Inhibition of in vitro angiogenesis by PI-88 alone or in combination with steroids

*FERRO V (1), CHAPMAN P (2), MCDONALD L (1), HAMMOND E (1), LI C (1)*
*(1) Progen Industries Ltd, Brisbane*
*(2) OE Ireland Ltd, Dublin*

**Purpose** Pathologic angiogenesis is associated with major causes of human blindness such as diabetic retinopathy and age-related macular degeneration (AMD). Angiogenesis is a complex process involving multiple heparan sulfate (HS)-binding growth factors such VEGF and FGF-2 and the HS-degradative enzyme heparanase. The Phase II oncology candidate PI-88 is a potent inhibitor of the actions of these proteins and its activity was compared to known inhibitors. Its ability to inhibit in vitro angiogenesis alone or in combination with steroids was assessed.

**Methods** PI-88 was tested for its ability to inhibit heparanase, to bind to angiogenic growth factors (VEGF, FGF-1, FGF-2) and to inhibit in vitro angiogenesis in a Matrigel tubule formation assay.

**Results** PI-88 showed good activity in all assays compared with known antiangiogenic agents such as suramin, pentosan polysulfate and steroids such as triamcinolone. PI-88 inhibited tubule formation in a Matrigel assay when used alone or in combination with a steroid. The results indicate that PI-88 has potential as a treatment for ocular diseases involving angiogenesis such as diabetic retinopathy and AMD, either as a monotherapy or in combination with a steroid.

**Conclusion** PI-88 is a potent inhibitor of various angiogenic factors and has potential as an ocular neovascularization inhibitor either alone or in combination with a steroid where synergistic activity is predicted. Combination therapy would allow lower steroid dosing and avoid the side effects which currently limit the chronic use of steroids in ocular therapy. [PI-88 has been shown to be effective in combination therapy in oncology and the multiple pathways of angiogenesis may similarly be best blocked by combination therapy].

■ **3435**

## Reduced retinal neovascularization in mice after intravitreal injection of monomeric soluble EphB4

*LANGE CAK (1), EHLKEN C (1), SCHAFFNER F (2), MARTIN G (1), HANSEN LL (1), AUGUSTIN HG (2), AGOSTINI HT (1)*
*(1) University Eye Hospital, Freiburg*
*(2) Tumor Biology Center, Freiburg*

**Purpose** Eph receptors and their membrane-tethered ephrin ligands comprise the largest family of receptor tyrosine kinases and are involved in the projection of growing axons. Studies on mice have revealed an important function of EphB4 and its ligand ephrinB2 during vascular development in embryonic life. Recent studies showed that soluble monomeric EphB4 inhibits angiogenesis and tumor growth in vitro. To investigate these effects in vivo we injected soluble monomeric EphB4 in the vitreous of mice using the murine model of oxygen-induced retinopathy (OIR).

**Methods** Retinal neovascularisation (RNV) in the OIR-model was induced by exposing a total of 26 mice (C57BL/6J) to 75% oxygen from postnatal day 7 (p7) to p12. On p12 they were returned to room air. Monomeric sEphB4 (100µg/µl) was injected in the right eye and buffer solution in the left eye. RNV was visualised by perfusion with fluoresceine-dextran on p17. Retinal whole mounts were prepared and their blood vessel patterns were quantified using a scoring system adapted from Higgins et al.

**Results** Intravitreal injection of monomeric sEphB4 treatment reduced the retinopathy score compared to the individual control eyes (n=26). Wilcoxon signed rank test analysis of the RNV scores showed a statistically significant difference ($p<0.01$).

**Conclusion** After having shown previously that dimeric EphB4 increases retinopathy in the OIR we now demonstrated an angioinhibitory effect after intravitreal injection of monomeric EphB4. This is in accordance with in vitro results in a tumor assay. Thus, monomeric EphB4 might be a therapeutic option in the treatment of angioproliferative retinopathy in men.

■ **3436**

## PI-88: a potent angiogenesis inhibitor with potential for treatment of back-of-the-eye diseases

*CHAPMAN P (1), FERRO V (2), PARISH C (3), FREEMAN C (3)*
*(1) OE Ireland Ltd, Dublin*
*(2) Progen Industries Ltd, Brisbane*
*(3) Immunology and Genetics, John Curtin School of Medical Research, Canberra*

**Purpose** To provide an overview of the antiangiogenic drug candidate PI-88 which is currently in multiple Phase II clinical trials for cancer and has potential applications as a regulator of ocular angiogenesis and inflammation.

**Methods** Review of the literature and recent clinical and non-clinical data.

**Results** PI-88 is a sulfated oligosaccharide heparan sulfate mimetic that shows promise as an inhibitor of tumor angiogenesis. It is currently under evaluation in Phase II clinical trials in several different cancers, is well tolerated and shows low systemic toxicity. It exerts antiangiogenic effects by antagonizing the interactions of key angiogenic growth factors (VEGF, FGF-1 and FGF-2) and their receptors with cell surface heparan sulfate (HS). PI-88 is also a potent inhibitor of heparanase, an enzyme that plays an important role in angiogenesis and vascular remodelling by cleavage of the HS side chains of proteoglycans in the extracellular matrix and basement membranes and thus liberating bound angiogenic growth factors. The multiple modes of antiangiogenic and anti-inflammatory activity displayed by PI-88 make it a good candidate for the treatment of ocular neovascularization associated with diabetic retinopathy and age-related macular degeneration (AMD). Direct intraocular applications may include use as an adjunct to pars plana vitrectomy (PPV) and glaucoma filtration surgery when used in irrigation solutions. Direct intravitreal injection for intraocular inflammation in diabetic macular oedema (DME) or non infectious uveitis may be appropriate.

**Conclusion** PI-88 is a potent inhibitor of angiogenesis with multiple modes of action and low systemic toxicity with potential for the treatment of diabetic retinopathy, AMD and DME.

■ 3451

## A complete, open-access, evidence-based and objective system of analysis of posterior capsular opacification

*ASLAM TM (1), PATTON N (2), DHILLON B (2), GRAHAM J (3)*
*(1) Royal Eye Hospital, Manchester*
*(2) Eye Pavilion, Edinburgh*
*(3) Imaging Science, Manchester University, Manchester*

**Purpose** To develop a computerised system of analysis of digital images of posterior capsule opacification(PCO) that is evidence based, objective and freely available. It should incorporate and improve on the best features of the currently available systems and yet be accessible to all. Evidence for the reliability and validity of the developed system is presented.

**Methods** The system of PCO analysis was developed considering current published evidence on visual significance of PCO and additional investigative analysis of PCO images. The program incorporates the automatic merging of images, after registration, to remove flash artefacts. It involves several essential preprocessing steps and finally measures a texture-based entropy score weighted toward proximity to central areas of PCO . The entire system has been compiled for use on any PC running Windows. In order to assess validity, the systems ability to measure PCO progression is assessed along with the visual significance of its final computerised scores. Reliability of the system is also assessed.

**Results** The system runs successfully and is simple to use. Its compiled form works on any PC operating windows. Analyses of PCO by the system show an ability to detect early progression of PCO as well as detection of visually significant PCO. Images with no clinical PCO produce very low scores in the analysis. Reliability of the system of analysis is shown to be satisfactory.

**Conclusion** This paper presents a complete system of PCO analysis that is evidence based, objective and accessible . It incorporates the best features of current specialised systems and our own evidence-based modifications which substantially improve validity.

■ 3452 / 348

## Lens optical quality: the area under the modulation transfer function correlates with visual acuity

*SHAMS PN (1), DAVIES NP (1), WEALE RA (2)*
*(1) Ophthalmology. Chelsea and Westminster Hospital, London*
*(2) Ophthalmology. University College London Hospital, London*

**Purpose** The OQUAL™ is a device that attaches to a slit lamp and projects a series of gratings of increasing periodicity into the eye. The 4th Purkinje image is captured with a digital camera. The resulting image is analysed to derive the modulation transfer function (MTF) of the lens as a measure of its optical quality.

**Methods** In this study 33 patients with increasing grades of cataract and no other pathology were imaged and their visual acuity measured. The MTF was calculated from the grating image using a Fourier transform method described previously (EVER 2003) and the total area under the MTF calculated. This was then compared with the visual acuity.

**Results** There is a significant correlation of area under the MTF with visual acuity measured as a Snellen fraction. The MTF area decreased linearly as a function of decreasing Snellen fraction as cataract worsened. The correlation coefficient was 0.73 giving r2= 0.58 ($p<0.0001$).

**Conclusion** The area under the MTF as measured using the OQUAL gives an independent measure of lens quality that correlates with the visual acuity. In cases of reduced visual acuity due to both cataract and macular disease, the OQUAL can be used to separate the effect of cataract on the visual acuity, to ascertain whether cataract surgery would be likely to be of benefit.

■ 3453 / 349

## 3D images of cataract surgery: a new telemedicine and didactic tool

*LIBONDI T (1, 2, 3), CRICELLI R (1), CAPUTO S (1), ZEPPA L (4), GIACOIA A (1), COLONNA G (2)*
*(1) Dept of Ophthalmol. II Univ, Napoli*
*(2) CRISCEB (Center for Res. in Comp.Sci. and Biotech.) II Univ, Napoli*
*(3) Medical Services sas, Napoli*
*(4) UOC Ocul. Osp S. Giuseppe Moscati, Avellino*

**Purpose** The importance of stereo viewing in microscopic ophthalmic surgery is well known. Surgical images are nowadays bidimensional, lacking the third dimension. We wanted to test the possibility to obtain and store stereo images obtained at the microscope during cataract surgery.

**Methods** A Zeiss OPMI 1 surgical ophthalmic microscope was equipped with two C-mount adaptors in such a way that each of them was able to capture left and right images of the surgical field as seen by the surgeons. Two Olympus Camedia C-4040 Zoom digital cameras were used to simultaneously acquire left and right images. They were stored in JPEG format in a dedicated workstation, and thereafter processed and rendered by using Stereographics-3D (TM) software and Crystall Eyes 3 goggles on a high definition monitor.

**Results** High definition stereoimages of the anterior segment of the eye as seen at the microscope were acquired during routine surgery for cataract (phacoemulsification and IOL implantation). The main problems encountered with our setting were proper focusing and exact alignment of the two different images. The approx. size of a single left or right JPEG image was about 1 Mb, thus permitting a rather easily storage and forward through the net. By viewing them with stereo rendering, the spatial relationships and the relative depth of the different structures could be clearly appreciated, as opposed to the single left and right images when seen separately.

**Conclusion** It was possible to take, store, send and render 3D images as taken at the microscope during a phacoemulsification procedure for cataract. The addition of stereopsis added reality to the images, as compared to the single flat bidimensional image.

■ 3454

## Selecting patients undergoing cataract surgery according to their sensitivity to pain

*GOMBOSS K (1), JAKUBOVITS E (2), SALACZ GY (1), NAGY ZZS (1)*
*(1) Departement of Ophthalmology, Semmelweis University, Budapest*
*(2) Department of Physiology and Morfology, Semmelweis University, Budapest*

**Purpose** To identify parameters which would help to categorise cataract patients preoperatively according to their sensitivity to pain, and to identify the most suitable praemedication and/or anaesthetic method.

**Methods** 116 normal cataract patients were enrolled in our study. To randomise the anaesthetic method, we alternated the methods daily (using retrobulbar anaesthesia one day and topical anaesthesia the next). Surgeons were divided into two groups (more than 10 operations/week or less). Psychological state of patients was measured by the State-Trait Anxiety Inventory (STAI) test and face scale test before and after surgery. Blood pressure, heart rate, blood oxygen saturation and sign of pain or discomfort was recorded at various stages during surgery. Preoperative and postoperative white blood cells, glucose, cortizol, adrenalin and noradrenalin blood levels were compared . Statistical analysis was performed by mean level comparison and logistic regression.

**Results** Significant difference was found between the type of anaesthesia and the pain experience during the procedure. There was no significant correlation between the presence of pain and psychological states, catecholamine levels, systolic blood pressure, the given surgeon's experience and the age of patients. However by analysing this data using logistic regression, the authors were able to estimate 98 percent probability whether pain could be expected during the procedure.

**Conclusion** We concluded that by using these statistic methods we can select the most vulnerable patients and the most appropriate type of praemedication and/or anaesthesia in their cases.

## ▪ 3455 / 350
## Variation in the theatre team and its impact on the outcome of complicated cases

*BAYLIS OJ, ADAMS W, FRASER SG*
*Sunderland Eye Infirmary, Sunderland*

**Purpose** The outcome of complicated (i.e. posterior capsule rupture) cataract surgery partly depends on how effectively the complication is dealt with. Rapid assessment and efficient management of the problem can make all the difference. This study was designed to investigate how the variations in experience of non-medical personnel in theatre could affect the outcome of a complicated case. For the purpose of the study we defined a good outcome as one in which the patient did not have to return to theatre for a further corrective procedure (e.g. further vitrectomy).

**Methods** 100 complicated cataract cases were identified. For each case, the experience of the scrub nurse and the cumulative experience of the nursing team present were recorded. Any procedures that the patient underwent following the initial surgery were also recorded.

**Results** 7 out of 50 (14%) complicated cases involving scrub nurses with 10 or more years experience required further procedures. 10 out of 32 cases (31%) with scrub nurses with 7 or less years experience required further procedures. In the 'more experienced' cohort for cumulative experience, 8 further procedures were performed. In the 'less experienced' cohort, 14 were done.

**Conclusion** These results suggest that when cataract surgery is complicated by posterior capsule rupture, fewer patients need corrective surgery later when more experienced non-medical staff are present in theatre. Case mix for both groups was similar suggesting that this could be due to greater experience allowing a more rapid and effective response by the non-medical team, permitting the surgeon to rescue the primary procedure e.g. by completing the anterior vitrectomy or placing a sulcus lens. As far as we are aware this is the first time this has relationship has been found.

## ▪ 3456 / 351
## Intraoperative breakage of the mushroom manipulator tip during phacoemulsification

*PELOSINI L, RICHARDSON EC, GOEL R, HUGKULSTONE CE*
*Queen Mary's Hospital, Sidcup*

**Purpose** To report a series of three cases of breakage of mushroom nucleus manipulators during phacoemulsification performed by two different surgeons in the same Unit.

**Methods** The three reported incidents occurred to two different surgeons over a period of three months. Case 1: the mushroom manipulator tip was missing at the end of the phacoemulsification, the capsular bag was filled with viscoelastic and the broken tip was retrieved with capsulorhexis forceps.Case 2: at the end of a successfull phacoemulsification the second instrument was removed from the eye and the mushroom head broke off the stem, remaining embedded on the side port. The fragment was dislodged with viscoelastic and removed using suction of a lacrimal cannula through the main wound. Case 3: the mushroom tip was noticed to be floating free in the capsular bag during the segments removal and was removed by gentle aspiration with a lacrimal cannula.

**Results** In all cases the breakage of the manipulator tip was detected at the end of nucleus phacoemulsification, the fragment was successfully retrieved and patients did not suffer any adverse effect. The examination of the instruments revealed significant wear around the stem, decreased thickness of the material and numerous surface marks by abrasive action.

**Conclusion** Intraoperative breakage of instruments during phacoemulsification may lead to severe complications, this is the first series of intraoperative macroscopic breakage of the same type of nucleus manipulator without ocular damage.We would recommend care to avoid inadvertent contact between instruments within the anterior chamber during phacoemulsification.

## ▪ 3457 / 352
## Effect of Sodium Hyaluronate (Ophthalin®) and Hydroxypropylmethylcellulose (HPMC-Ophtal®) on Corneal Endothelium, Central Corneal Thickness, and Intraocular Pressure after Phacoemulsification

*VOROS GM, RESSINIOTIS T, RAY-CHAUDHURY N, SUTHERLAND S, MITCHELL K, FIGUEIREDO FF*
*Royal Victoria Infirmary, University of Newcastle upon Tyne, United Kingdom., Newcastle upon Tyne*

**Purpose** To prospectively evaluate the effects of 2% hydroxypropyl-methylcellulose (HPMC-Ophtal®) and sodium hyaluronate 1% (Ophthalin®) on intraocular pressure, corneal thickness and endothelial cell loss in small incision cataract surgery with implant.

**Methods** 110 patients undergoing routine phacoemulsification with implant received either 2% hydroxypropylmethylcellulose or sodium hyaluronate 1% as ophthalmic viscosurgical device. Pre- and post-operative slit lamp examination, intraocular pressure measurement (pre-operatively and at 1-4 hours, 1 day and 7 days post-operatively), ultrasonic pachymetry (pre-operatively and at 1 week, 4-6 weeks, and 12 weeks post-operatively) and corneal endothelial cell count (pre-operatively and 12 weeks post-operatively) were performed. Data was analysed using two way analysis of variance.

**Results** Intraocular pressure was significantly lower in the Ophthalin® group at 1 day post-operatively, while no significant difference was found between the two groups on the 1-4 hours and 7 days examination. Post-operative central corneal thickness was not significantly different between the two groups. The mean cell density demonstrated a significant fall of 11.76% for Ophthalin® and 4.27% for HPMC-Ophtal® at twelve weeks post-operatively, the difference between the two being significant (p=0.009).

**Conclusion** 2% hydroxypropylmethylcellulose, compared with sodium hyaluronate 1%, is superior in protecting the corneal endothelial cells, has the same effect on central corneal thickness, and is associated with slightly higher intraocular pressure one day post-operatively.

## ▪ 3458
## Physiopathology of the lens capsule after implantation of an intraocular lens

*DE KEYZER KHMH, LEYSEN I, TASSIGNON MJ*
*Department of Ophthalmology, Antwerp*

**Purpose** To evaluate the difference in epithelial cell growth between lens-in-the-bag implantation versus bag-in-the-lens implantation in an in vitro capsular bag model.

**Methods** 36 capsular bags of human donor eyes were put in culture for 4 weeks, divided in 3 groups: without IOL, with Morcher 92S (lens-in-the-bag) insertion and with Morcher 89A (bag-in-the-lens) implantation. Each of the groups were divided in 4 subgroups by administering them either only standard medium with calf serum, or enriched with one of the following growth factors: FGF, TGF-beta and IL 6. All capsules were evaluated by fluorescence microscopy.

**Results** There was a remarkable difference in cell growth between the 3 groups. Bag-in-the-lens implantation was highly effective in restricting lens epithelial cell (LEC) proliferation in the remaining lens bag of human donor eyes. Lens-in-the-bag implantation however showed extensive proliferation and differentiation of LEC.

**Conclusion** Bag-in-the-lens implantation was highly effective in restricting LEC-proliferation in the remaining lens bag of human donor eyes.

## ▪ 3461
## Introduction to measurement of accommodation

*GLASSER A*
*Optometry, Houston*

**Purpose** Accommodation is a dioptric change in power of the eye. Although accommodation is usually measured clinically with a subjective push-up test, this overestimates the true accommodative response and is inadequate to unequivocally demonstrate accommodation.

**Methods** Several methods to both stimulate and measure accommodation exist. Low concentrations of pilocarpine administered topically produce a direct contraction of the ciliary muscle and an ensuing involuntary accommodative response. Real, self illuminated targets viewed binocularly at far (6 meters) and at various near distances present compelling stimuli that contain blur, convergence and proximal cues. Far targets viewed through minus powered trial lenses present blur cues. The accommodative response can be measured objectively with static refractometers (Hartinger coincidence refractometer), autorefractors (Grand Seiko WR5500K) and wavefront aberrometers (TRACEY iTrace).

**Results** The subjective push-up test overestimates the true accommodative amplitude relative to objective measures. Pharmacologically stimulated accommodation occurs over 30 minutes and the response amplitude depends on iris color. An accommodative response can be elicited when compelling blur and/or proximity cues are presented. A variety of clinical instruments are available for objective measurement of accommodation.

**Conclusion** Accommodation can and should be measured objectively to unequivocally demonstrate if accommodation occurs. A variety of different methods to both stimulate and measure accommodation are available.

## ▪ 3462
## Optical techniques to measure natural accommodation

*SCHAEFFEL F*
*University Eye Hospital, Section Neurobiology of the Eye, Tübingen*

**Purpose** Although it is possible to observe biometrical changes in lens geometry or the ciliary body during accommodation by Scheimpflug imaging, MRI, or high resolution ultrasonography, one can never be sure about the resulting dioptric changes. Furthermore, since natural accommodation may be different from artifically stimulated accommodation, techniques that can measure natural accommodation are preferred.

**Methods** Techniques are described that measure accommodation based on Scheiner's principle, direct measurement of retinal image contrast, automated dynamic retinoscopy, retinal image size, photorefraction and wavefront analysis.

**Results** Most of the measurements principles have been developed to permit dynamic recordings of refraction. They still vary (1) with regard to their dioptric resolution, (2) the possibilities for calibration, (3) the possibility to measure both eyes at the same time, and (4) to record also pupil size and the direction of gaze.

**Conclusion** Measurement techniques that work from a distance, under open field viewing condiitions, are particularly valuable. A further advantage is if they can record the refractions of both eyes, pupil size and convergence.

## ▪ 3463
## Accommodation Measurement with Shin-Nippon Autorefractors

*WOLFFSOHN JS*
*Life and Health Sciences, Aston University, Birmingham*

**Purpose** To provide an overview on how to measure accommodation clinically with Shin-Nippon autorefractors and to examine their potential to assess the dynamics of ocular accommodation.

**Methods** Shin-Nippon autorefractors, also marketed under Grand Seiko, use infrared light reflected off the retina to assess the ocular refracting power of the eye. As with the extensively utilised Canon R-1 autorefractor, they offer an objective measurement of refractive error/ocular accommodation, and an open and binocular field of view. However, unlike the Canon R-1 which measured the overall intensity of infrared light reflected from the retina, the Shin-Nippon autorefractors project a pattern (ring or lines) of light and image analyse the change in shape after it has been reflected through the ocular refracting elements. Measurements can be made with pupil sizes as small as 2-3mm.

**Results** Static measurements of refractive error/ocular accommodation can be made in approximately 1 second by the instruments. However, with the SRW-5000 and FR-5000 the infrared light can be switched permanently on and the video image tapped to be analysed by an external computer at up to 60Hz. This allows the dynamics of accommodation to be assessed and is fairly robust to small eye movements and focusing adjustments. However, the newer model (NVision-K, SRW5001) has had an autokeratometer added and the refractive video output can no longer be tapped.

**Conclusion** Shin-Nippon autorefractors offer the ability to provide robust, objective measures of ocular accommodation at up to 60Hz while the patient maintains an open field of view.

## ▪ 3464
## Wavefront Measurement of Accommodation

*LOPEZ-GIL N*
*Fisica. Grupo de Ciencias de la Visión, Murcia*

**Purpose** Accommodation modifies ocular wavefront which can be measured objectively by means of aberrometers. Our purpose is to show how accommodation can be measured from wave-front outcomes; to present the changes in wavefront error that can be typically found during accommodation; and to show the potential use of this technique to obtain objective measures of accommodation and pseudophakic accommodation in subjects wearing multifocal and accommodative intraocular lenses.

**Methods** For the last three years, a few aberrometers have made it possible to stimulate the accommodation by positioning optically a stimulus at different distances, and to obtain accurate and objective measurements of the accommodation response. An objective measure of accommodation can be obtained by computing specific metrics from the resulting Zernike coefficients. In particular, a combination between defocus and spherical aberration is related to the subject's accommodation state. The effect of accommodative miosis is also analyzed.

**Results** Results obtained in several studies show that ocular wavefront aberrations change with accommodation. Although those changes exhibit inter-subject variability, there is a significant decrease in spherical aberration with increasing accommodation in young subjects that could influence the lag of accommodation. Recent measurements obtained with an Imagine Eyes irx3 aberrometer have shown that the change in ocular aberrations during accommodation is different in the young (pre-presbyope) and the old (presbyope) eye. Accurate measurements on intraocular implants (monofocal, multifocal and pseudo-accommodative) are also presented.

**Conclusion** Dynamic aberrometry is a valuable technique to investigate accommodation and evaluate various presbyopic corrections modalities.

■ **3465**

## Hands-on practice: Accommodation measurement

*GLASSER A, SCHAEFFEL F, MICHAEL R, LOPEZ-GIL N, WOLFFSOHN JS*

**Purpose** To allow audience participants to gain hands on experience in objective measurement of accommodation.

**Methods** We anticipate that several autorefractors, wavefront aberrometers and other commercially available clinical instruments will be on display to do objective accommodation measurements. The session speakers will interact with audience participants to demonstrate and explain how accommodation can be measured objectively with these instruments.

**Results** The experience will provide participants with hands on experience in objective accommodation measurement with several commercially available clinical instruments.

**Conclusion** Several commercial instruments are available for objective accommodation measurements. These can readily be introduced in clinical practice to do objective accommodation measurements.

## 4111
### How to choose a keratoprosthesis when you need one

*LIU C*
*Sussex Eye Hospital, Brighton*

**Purpose** A keratoprosthesis (KPro, artificial cornea) may be required for visual rehabilitation of cornea blindness, or for vitreo-retinal surgery in the presence of corneal opacification. Only the former indication will be discussed. Whilst there is a desire to replace cadaveric corneal grafting altogether, no existing device works better than conventional keratoplasty in low risk cases. Keratoplasty is complicated by astigmatism, immunological rejection, the possibility of transmission of infection from donor to host, and world shortage of donor material. However, it is still more reversible and poses less threat to ocular integrity than any keratoprosthetic device.A KPro should therefore only be considered for high risk cases: repeated corneal graft failure, the highly vascularised cornea and severe ocular surface disease. Whether a corneal graft should be attempted in the first place, or whether a repeat corneal graft should be attempted before considering a KPro, depends on whether there is reasonable prospect of success, and whether any immunosuppression regime will be tolerable.The choice of device is then governed by how hostile the ocular environment is (including dryness, lid or blink deficiency), laterality of disease, and availability of expertise in the locality. The author will present the range of devices available, and when they should be used. Follow up of KPro patients is life-long so keratoprosthetic centres should be strategically placed in different parts of the world. Referring corneal surgeons and external eye disease experts then need to be aware of the attributes of available devices, refer appropriately, and enter into shared care with a KPro centre.

## 4112
### Update on Brighton OOKP results

*AINSWORTH G (1), PAUL B (1), TANDON R (2), HEROLD J (3), THORP S (3), LIU C (1)*
*(1) Sussex Eye Hospital, Brighton*
*(2) RP Centre for Ophthalmic Science, New Delhi*
*(3) Royal Sussex County Hospital, Brighton*

**Purpose** To report updated Brighton OOKP results.

**Methods** Case note review of 35 consecutive cases of OOKP surgery at the Sussex Eye Hospital Brighton. Presenting diagnoses, pre- and post-OOKP visual acuity, and complications were recorded.

**Results** Demographics: 21 male. 14 female (n=35). Mean age at stage 2: 50.6 years (range 19-87). Presenting diagnoses: 15 Stevens Johnson Syndrome, 5 chemical injury, 6 ocular cicatricial pemphigoid, 4 miscellaneous dry eye diseases, 1 trachoma, 1 linear IgA disease, 1 ectodermal dysplasia, 1 post-bomb blast, 1 congenital trigeminal nerve hyoplasia.34/35 proceeded through stage 1 and stage 2 (1 developed at total retinal detachment between stage 1 and stage 2). Mean time interval between stage 1 and 2: 19.8 weeks (range 9-84).Pre-op visual acuity: PL (15), HM (15), CF (5). Post op visual acuity at last follow up: 6/5 (4), 6/6 (2), 6/9 (5), 6/18 (3), 6/24 (1), 6/36 (2), <6/60 (2), HM (4), CF (1), PL (3) and NPL (6) (2 patients required subsequent evisceration).Mean follow up: 34.7 months (range 1-96)There were 3 allografts. 7 exhibited clinical resorption of OOKP lamina. 2 subsequently required repeat OOKP surgery.Post-operative complications (episodes, not eyes): 5 retro prosthetic membranes, 4 retinal detachments, 3 vitreous haemorrhages, 3 extrusions of lamina, 2 infections of buccal membrane graft, 2 endophthalmitis, 1 expulsive haemorrhage and 1 epiretinal membrane.

**Conclusion** OOKP remains a valuable procedure in the restoration of sight in severely damaged dry ocular surface diseased eyes when conventional surgery is doomed to failure. It is however a procedure which carries significant risk for further surgery and potential blindness.

## 4113
### Results of the champagne-cork keratoprosthesis: preliminary data from a second centre in India

*TANDON R (1), SHARMA N (1), VAJPAYEE RB (1), GARG SP (1), SINGH I (2)*
*(1) RP Centre for Ophthalmic Sciences, All India Institute of Medical Sciences (AIIMS), New Delhi*
*(2) Daljit Eye Hospital, Amritsar*

**Purpose** To report the results of implanting the Daljit-Worst Champagne cork keratoprosthesis at a second surgical centre in India.

**Methods** Prospective, non-comparative case series. Dr Indu Singh from Amritsar imparted the training for implantation of the champagne cork keratoprosthesis. Dr Radhika Tandon at the R P Centre, AIIMS performed surgery with the help and support of colleagues. Informed consent was taken and the surgical and follow up protocol as approved by the ethics committee was followed. Results were noted as per the standard Performa devised and presented at EVER 2004. Preliminary data is being presented.

**Results** A total of 5 patients were recruited for the study and operated over a period of 4 months. The age ranged from 35years to 72years, mean 51.4y. There were 4 males and 1 female. All were bilaterally blind with primary ophthalmic disease associated with high risk for graft failure. All had a history of previous failed corneal grafts in one or both eyes. All patients had moderately dry eyes with Schirmer ranging from 2-8. Primary ocular disease was conjunctivo-corneal scarring from chemical burns in four patients and ocular cicatricial pemphigoid in one. One patient had had a retinal detachment after a prior graft for which VR surgery was done and which was followed by corneal decompensation. One patient had pre-existing glaucoma. Follow up ranged from 8mths to 1 year. All patients regained their visual potential. Two patients had very poor visual potential due to previous retinal detachment and glaucoma and had only counting fingers vision. The other tree patients had a vision of 6/36, 6/24 and 6/18 with +7.0D, +8.0D and +10.0D correction. One patient developed miliary tuberculosis during follow up and subsequently the operated eye developed cilio-choriodal detachment and vision dropped to counting fingers. There was no retinal detachment and no evidence of aqueous leak or endophthalmitis. There was no improvement with conservative therapy in the form of topical steroids and cylcloplegics, so silicone oil was injected into the vitreous cavity. Choroidals resolved and vision improved to 3/60 and maintained till last follow up 1 year of surgery.

**Conclusion** The champagne-cork KPRo gives good visual results in appropriately selected patients. The surgery can be performed at tertiary care centres by a team trained to perform the surgery and who are also competent enough to handle the post-op care and complications. Preliminary data shows that careful follow up over a longer period is required to correctly evaluate retention and long term success.

## 4114
### Imaging of Osteo-odonto-keratoprosthesis (OOKP) by High Resolution Computed Tomography (HRCT) after stage 1 and prior to Stage 2

*TANDON R (1), SHARMA R (2), ROYCHOWDHARY A (3), VASHISHT N (1), SHARMA N (1), LIU C (4)*
*(1) RP Centre for Ophthalmic Sciences, All India Institute of Medical Sciences (AIIMS), New Delhi*
*(2) Department of Radiodiagnosis, AIIMS, New Delhi*
*(3) Department of Dental and Maxillofacial Surgery, AIIMS, New Delhi*
*(4) Sussex Eye Hospital, Brighton*

**Purpose** To describe our experience of using HRCT in imaging OOKP as an aid to monitoring the site, dimensions and orientation of the implant in the lower lid after stage 1 and just before performing stage 2.

**Methods** HRCT has been performed on our first patient of OOKP surgery seven days after stage 1b i.e. implantation of the crafted OOKP lamina in the submuscular pouch of the lower lid. The radiologist was masked to the actual measurements taken at the time of surgery. HRCT was then repeated 2 days before stage 2. The surgeon was masked to the radiological measurements before the surgery. The scans were performed as 2/2mm sections and the measurements were made by 3-D reconstruction of the images. A measuring scale was superimposed on the images by the computer to take the necessary measurements following standard techniques. The repeat scans were performed ensuring that the patient was in the same position as the baseline scan to avoid any confounding effect due to positioning or windowing errors. The manual measurements at surgery were made with a combination of a sterile mm scale and vernier calipers and were verified by 2 other observers. All these measurements assessing the dimensions of the OOKP lamina were then compared.

**Results** A 28-year-old bilaterally blind female patient of Stevens Johnson Syndrome with severe dry eyes underwent OOKP surgery in stages. As this was to be the first case operated by the surgical team in Delhi, stage 1a was performed and after demonstrating a satisfactory result we proceeded to harvesting, fashioning and implanting the OOKP lamina in stage 1b. Measurements taken at stage 1a were a maximum length of 15mm, width of 12mm and thickness of 3-4mm. Measurements taken 1 week later by HRCT were 10.7mm, 5mm and 3.5mm respectively. The surgeon was not expecting any change at such a short interval so the measurements were not analysed at that time. Repeat measurements taken by HRCT after 15 weeks were 13.9mm, 5.8mm and 3.1mm respectively. Manual measurements taken during surgery 2 days after HRCT were 20mm, 16mm and 4mm. Surgery was technically successful and patient regained a vision of 6/18 with +3.0D, N6 with +6.0D.

**Conclusion** HRCT did serve as a guiding aid for the surgical team for their first patient. The scan performed just before stage 2 was reassuring in confirming that the position, orientation and general dimensions were stable. The measurement of width was very unreliable by HRCT in comparison with manual values as performed in this case.

■ **4115**

## Boston Keratoprosthesis after Herpetic Keratitis and in Aniridia

*KHAN BF, DOHLMAN CH*
*Cornea and Refractive Surgery, Massachusetts Eye and Ear Infirmary, Boston, MA*

**Purpose** Herpetic keratitis and aniridia have the reputation of having poor prognosis for keratoprosthesis (KPro). Here a small patient series with these diagnosis are reviewed

**Methods** By chart review 8 patients with herpetic history (7 simplex and 1 zoster), as well as 3 patients with aniridia, were identified. Vision was recorded as preoperative, best post operative and last followup. Complications are described

**Results** In the 8 herpetic patients, mean penetrating keratoplasties (PK) were 2.7 per patient. Vision was pre op from light perception (LP) to count fingers (CF), best post op from 20/30 to 20/400 and last followup from 20/40 to no light perception (glaucoma). Pre KPro conditions included glaucoma (5), AMD (3), CME (2), scleral buckle (1). Post KPro complications were retro KPro membrane (3) and fungal keratitis (1). Two cases of HSV had active inflammation and ulceration which rapidly quieted after surgery. The 3 aniridic patients also had glaucoma, and one had 2 PK and 2 none. Last vision ranged from hand motions to 20/60. One tissue melt was repaired, but without removal.

**Conclusion** The KPro maintained stability of the eyes without any extrusion. All the implantations resulted in totally quiet eyes. Vision was limited due to co-morbid conditions, particularly glaucoma. Thus, the Boston KPro seems well tolerated in herpetic keratitis and in aniridia.

■ **4116**

## Blockage of Glaucoma Shunts in the Boston Keratoprosthesis: Relationship to Lens Status

*COLBY KA (1, 2), DOHLMAN CH (1, 2)*
*(1) Massachusetts Eye and Ear Infirmary, Boston, MA*
*(2) Harvard Medical School Department of Ophthalmology, Boston, MA*

**Purpose** Glaucoma is a significant issue for KPro patients. In a recent multi-center study of 106 Boston KPro patients, two-thirds had pre-existing glaucoma. Over 50% had glaucoma shunt placement before, during or after KPro insertion. Blockage of the intraocular tube, a recognized complication of glaucoma shunts, is particularly problematic in KPro patients. Methods to prevent this complication will be reviewed.

**Methods** Chart review of 17 Boston KPro patients with experimental double-tubed Ahmed shunts in which the distal tube drains to the inferior fornix (6 cases) or the maxillary sinus (11 cases).

**Results** Vitreous incarceration into the intraocular end of the shunt occurred in 4 cases, with increased IOP due to tube obstruction. Nd:YAG laser vitreolysis restored shunt function in 1 case. Diode laser treatment was required to control IOP in the remaining 3 cases. Three patients were aphakic. One patient was pseudophakic, but had extensive vitreous prolapse around the posterior chamber intraocular lens at the time of KPro implantation. No cases of vitreous incarceration were noted in cases where the posterior capsule was intact.

**Conclusion** Aphakia and breach of the posterior capsule are risk factors for vitreous incarceration and failure of Ahmed glaucoma shunts in Boston KPro patients. Intraocular lenses act as a barrier to prevent forward movement of vitreous. To reduce the risk of this complication, we suggest the following: first, stable intraocular lenses should be left in place and used with a pseudophakic KPro. Second, a plano posterior chamber lens should be placed during concomitant cataract extraction and used with an aphakic KPro. Third, consideration should be given to implanting a plano IOL in aphakic patients receiving a Boston KPro.

■ 4121

## Applanation Tonometry: its use, corneal sources of error, and clinical implications

*DAYANıR V*
*Adnan Menderes University, School of Medicine, Department of Ophthalmology, Aydin*

**Purpose** The purpose of this talk is to discuss the pitfalls inherent to the use of Goldmann applanation tonometry.

**Methods** Working principle and proper use of Goldmann applanation tonometry will be revisited. Various sources of error originating from the eye, and pathogenic and iatrogenic eye diseases will be discussed. Particular emphasis is going to be given to the relationship between intraocular pressure and corneal thickness.

**Results** Results of different clinical papers will be discussed.

**Conclusion** The influence of corneal thickness on Goldmann applanation tonometry measurement had been questioned more often after the results of Ocular Hypertension Treatment Study had been published. Accordingly ocular hypertensive people with thinner corneas were associated more often with the development of glaucoma. One possible explanation is that people with thinner corneas actually have higher intraocular pressure than measured with Goldmann applanation tonometry pointing to a flaw of the measurement technique. Thus the throne of the Goldmann applanation tonometry as the 'gold standard' of intraocular pressure measurement is being shaken. We will try to sum up the data leading to these conclusions and try to provide solutions to the problem.

■ 4122

## Adjusting Goldmann Tonometry for corneal sources of error

*KUBE T*
*University Eye Hospital, Freiburg*

**Purpose** To explain the different rationales for adjusting Goldmann Tonometry data for corneal sources of error

**Methods** First, Goldmanns original theorem for intraocular pressure measurements and its inherited limits will be discussed in detail. Common corrective formulas for astigmatism and corneal thickness, published or used in commercially available devices, will be presented an discussed thoroughly.

**Results** As Goldmann tonometry data are only correct if the assumptions of Goldmann are fulfilled, alternative formulas and techniques have been developed to give the ophthalmologist more precise IOP readings.An overview of all published data comparing methods and/or corrective formulas is given.

**Conclusion** Goldmann tonometry data, corrective formulas and their crucial limits seem to demand an alternative method for precise IOP measurement, especially in abnormal or alterated corneas.

■ 4123

## Dynamic contour tonometry: is the "gold standard" for intraocular pressure measurement changing?

*PACHE M*
*University Eye Clinic, Freiburg*

**Purpose** The PASCAL dynamic contour tonometer (DCT) is a novel device designed for intraocular pressure (IOP) measurements assumed to be largely independent of corneal properties. We compared DCT with Goldmann applanation tonometry (GAT) in eyes with normal corneas.

**Methods** We prospectively measured IOP using DCT and GAT in random order in 100 right eyes of 100 subjects (M:F= 46:54; mean age: 42 ± 19 SD, range 23-88 y). The agreement between the two methods was tested by Bland-Altman-Analysis. In 23 eyes with a difference DCT-GAT of > 2 mmHg, measurements were repeated after ≥ 3 weeks. The IOP-values of the fellow eyes in this subgroup were assessed in parallel.

**Results** Bland-Altman-Analysis [2(GAT-DCT)/GAT+DCT] demonstrated a mean difference of -1 mmHg (CI: 1,2 mmHg). The 95%-confidence interval of the difference GAT-DCT was 4,3 mmHg. The difference GAT-DCT was -1,0 ± 0,25 SEM mmHg for all 100 eyes, and -2,5 ± 0,35 SEM mmHg for the subgroup of the 23 right eyes and -1,9 ± 0,42 SEM mmHg for the fellow eyes, respectively.

**Conclusion** In our study, DCT and GAT show acceptable agreement. In cases with higher discrepancies between the two methods, the difference is reproducible even in both eyes. We therefore assume that this difference is not accidental, but related to structural corneal properties which must be further discussed.

■ 4131

## Macular Pigment Measurement Using Motion Nulling

*MELLERIO J (1), WEST P (2)*
*(1) Biosciences, University of Westminster, London*
*(2) Cambridge Research Systems, Rochester*

**Purpose** To optimise parameters of a motion-nulling CRT-based method of measuring macular pigment (MP) optical density.

**Methods** The motion-nulling technique of Anstis & Cananagh (1983) is used with subjects centrally fixating a CRT display whilst test fields are presented at eccentricities from zero to 8 degrees. In the test field a horizontal grating appears to move up or down depending on the relative luminance of the chromatic square wave grating component of the stimulus. The central field was a 1 degree circle, the remainder were quarter segment arcs to the right or left of, and concentric with, the fixation point. An eye tracking system ensured accurate fixation and a blocking filter separated the blue & red phosphor emissions into a component (blue) absorbed by the MP and a component (red) that was not. Parameters investigated included arc width, duration of test presentation, spatial frequency of test grating, frequency of cycling of grating components and contrast of the achromatic component of the Anstis grating.

**Results** Perception of movement was sensitive to spatial and cycling frequency of all of the grating components and to the contrast range of the achromatic component. Troxler and movement after effects were avoided by keeping the test field presentation to no more than 500 ms every 1.5 sec. Optimal values for each parameter varied from subject to subject but it was possible to specify a set of "general purpose" values suitable for measuring MP in a clinic.

**Conclusion** The use of a CRT-based motion-nulling technique with monitored eye fixation has been successfully achieved in an instrument suitable for laboratory or clinic measurement of MP profiles.

■ 4132

## Imbalance of Extracellular Matrix turnover in Age-related macular degeneration

*SIVAPRASAD S (1, 2), CHALI KY (3), LUTHERT PJ (3), BAILEY TA (2), CHONG NHV (1)*
*(1) King's College Hospital, London*
*(2) Cranfield BioMedical Centre, Bedfordshire*
*(3) Institute of Ophthalmology, London*
*(4) Kings College Hospital, London*

**Purpose** Dysregulation of extracellular matrix (ECM) turnover is thought to contribute to the pathogenesis of age-related macular degeneration. Elastin is a fibrous protein constituent of the ECM. The degradation of elastin is detected by the presence of serum elastin derived peptides (S-EDP) in circulation. Matrix metalloproteinases (MMPs) especially gelatinase A (MMP-2), gelatinase B (MMP-9) are important elastolytic enzymes. This study estimated S-EDP, MMP-2 and MMP-9 in subjects with age related macular degeneration (AMD) compared to age-matched controls in order to determine the correlation between these elastolytic enzymes and elastin degradation in various stages of the disease.

**Methods** Thirty-five subjects with AMD were classified into two groups: early age-related maculopathy (ARM) and neovascular AMD. The control group consisted of 10 age-matched subjects with no AMD. S-EDP, MMP-2 and MMP-9 levels in the serum of these subjects were estimated by competitive ELISAs.

**Results** S-EDP was significantly higher in AMD compared to controls. In addition, subjects with neovascular AMD had higher levels of S-EDP compared to those with early disease. Plasma levels of MMP-2 were not significantly different in the three groups. MMP-9 was significantly higher in the ARM and neovascular AMD group compared to control. The MMP-9 showed a positive correlation with S-EDP levels in the control group. However, the correlation decreased in the ARM group and there was no correlation in the neovascular AMD group.

**Conclusion** This study shows that an imbalance exists between the proteolyic enzyme MMP-9 and the elastin degradation peptides in AMD and this imbalance increases with disease severity.

■ 4133

## Low densitiy lipoprotein (LDL) and its oxidized form induce early age related macular degeneration characteristics in vitro.

*WELGE-LÜßEN UC, KOOK D, KAMPIK A*
*Department of Ophthalmology LMU Munich, Munich*

**Purpose** Recent studies have revealed an association between coronary risk factors and the appearance of age related macular degeneration (AMD). Early AMD is characterized by changes in the retinal pigment epithelium (RPE) like cell loss, advanced senescence, increased growth factors expression and extracellular matrix (ECM) accumulation. Similar mechanisms occur in atherosclerosis, in which uptake of oxidized low density lipoprotein (Ox-LDL) by macrophages via scavenger receptor involves the initial lesion. It has been previously shown that OX-LDL and scavenger receptors are also present in the RPE. Therefore we investigated the effect of Ox-LDL on the early cellular key events of AMD.

**Methods** Cultivated RPE cells from 5 human donors of the third passage were incubated with 10-100 µg Ox-LDL. Cell loss was investigated by life dead assay. For determination of advanced senescence beta galactosidase staining was used. The expression of the growth factor TGF-beta was analyzed by an ELISA assay. The induction of collagen type IV, laminin and fibronectin were quantified by western and northern blot analysis.

**Results** Ox-LDL markedly induced cell death of cultured RPE cells and accelerated the onset of RPE senescence, whereas LDL showed no effect. Expression of TGF-beta was increased by Ox-LDL (400 pg/ml +/- 45) and LDL (195 pg/ml +/- 24). Furthermore Ox-LDL induced ECM proteins (collagen type IV 190%, laminin 145 % and fibronectin 170 %) on the mRNA and protein level. LDL treatment showed similar results.

**Conclusion** OX-LDL and LDL seems to induce early cellular events of AMD in the RPE. Therefore it is likely, that optimisation of atherosclerotic risk factors could reduce the prevalence of early AMD.

■ 4134

## Global gene expression in normal RPE and in CNV-RPE determined by SAGE

*MARTIN G, AGOSTINI HT, HANSEN LL*
*University Eye Hospital, Freiburg*

**Purpose** Age-related macular degeneration (AMD) as the leading cause of blindness in elderly persons has been well documented at the clinical and histological level. But the underlying molecular mechanisms are almost unknown. Therefore, our experiments aim to get insight into the regulatory processes taking place in the retinal pigment epithelium (RPE) and resulting in the formation of choroidal neovascular membranes (CNV membranes).

**Methods** Determination of gene expression profiles from cell cultures of normal (ARPE-19) and CNV membrane-derived RPE (CNV-RPE) by serial analysis of gene expression (SAGE).

**Results** Data are compared to published expression profiles of normal RPE and used for finding genes differentially expressed in different RPE cell types. Interestingly, as we reported previously, vimentin is found in ARPE-19 and CNV-RPE but not in normal RPE.

**Conclusion** Comparison of the whole gene expression profiles shows that ARPE-19 are similar to normal RPE. CNV-RPE cells show more differences to normal RPE as it is expected according to their transdifferentiation state. Investigating the regulation of differentially expressed genes from normal and CNV-RPE provides access to the regulatory network of AMD.

## ▪ 4135
## In vitro AAV gene transfer optimisation on ARPE cells

*KOCH P (1, 2), DE GREEF S (1, 2), BRUYNS C (2), CASPERS L (1), TENENBAUM L (2), WILLERMAIN F (1, 2)*
*(1) Ophtalmologie, Brussels*
*(2) IRIBHM, Brussels*

**Purpose** RPE cell transplantation may be a possible treatment for many retinal diseases. However, several limitations, such as immune rejection, have been encountered in RPE cell transplantation research. These could be overcome by the use of gene transfer. We hereby propose to investigate the best serotype of adeno-associated virus (AAV) to lead to optimum ARPE cell infection and the best promoter for gene expression in vitro. We have finally illustrated our work with an example of anti-inflammatory gene transfer (SOCS1).

**Methods** The infection efficiency of AAV serotype 1, 2, and 5 on ARPE cells was compared using the eGFP reporter gene under the control of the CMV promoter, using three different multiplicities of infection (MOI). In a second step, different promoters (CMV, CAG, Tet-inducible) were evaluated. For all eGFP experiments, fluorescence was measured at day 2 and 5 by FACS. Finally, ARPE cells were infected by AAV2-CAG-SOCS1 and stimulated by IFNg before measuring MHCII expression by FACS.

**Results** We showed that at a MOI of 60, AAV serotype 2 vectors transduce up to 95% of ARPE cells which is 2- to 3- fold higher than the other serotypes. Fluorescence was detected by FACS at 2 and 5 days. The mean fluorescence intensity was 10- fold higher with CMV as compared with CAG and TetON promoters. Finally, SOCS1 gene transfer mediated by AAV2 in cultured ARPE inhibited IFNg-mediated MHCII expression.

**Conclusion** AAV2 is the best serotype to infect ARPE cells in vitro, whereas the CMV promoter is the most efficient to allow a strong gene expression in vitro. Anti-inflammatory gene transfer could be efficiently achieved using this technique. AAV might thus be of interest for manipulating the gene expression of RPE cells in RPE transplantation.

## ▪ 4136 / 445
## Multispectral Fundus Analysis

*CALCAGNI A (1, 2), STYLES IB (1), CLARIDGE E (1), ORIHUELA-ESPINA F (1), GIBSON JM (2)*
*(1) University of Birmingham - School of Computer Science, Birmingham*
*(2) Birmingham and Midland Eye Centre, Birmingham*

**Purpose** To generate a reflectance model of the fundus that allows an accurate non-invasive quantification of blood and pigments.

**Methods** A Monte Carlo simulation was used to produce a mathematical model of light interaction with the fundus at different wavelengths. The model predictions were compared with fundus images from normal volunteers in several spectral bands (peaks at 507, 525, 552, 585, 596 and 611nm). The model was then used to calculate the concentration and distribution of the known absorbing components of the fundus.

**Results** The shape of the statistical distribution of the image data generally corresponded to that of the model data; the model however appears to overestimate the reflectance of the fundus in the longer wavelength region.As the absorption by xanthophyll has no significant effect on light transport above 534nm, its distribution in the fundus was quantified: the wavelengths where both shape and distribution of image and model data matched (<553nm) were used to train a neural network which was then applied to every point in the image data. The xanthophyll distribution thus found was in agreement with published literature data in normal subjects.

**Conclusion** We have developed a method for optimising multi-spectral imaging of the fundus and a computer image analysis capable of estimating information about the structure and properties of the fundus. The technique successfully calculates the distribution of xanthophyll in the fundus of healthy volunteers. Further improvement of the model is required to allow the deduction of other parameters from images; investigations in known pathology models are also necessary to establish if this method is of clinical use in detecting early chroido-retinopathies, hence providing a useful screening and diagnostic tool.

## ▪ 4137 / 446
## Zinc in Drusen

*PETO T (1), LENGYEL I (2, 3), FREDERICKSON C (4), VAN KUIJK E (5)*
*(1) Moorfields Eye Hospital, London*
*(2) Institute of Ophthalmology, London*
*(3) Biological Research Centre, Szeged*
*(4) Neurobiotex, Galveston*
*(5) Ophthalmology and Visual Science, Galveston*

**Purpose** Abnormal zinc metabolism has been described in retinal disorders such as age-related macular degeneration (AMD). One of the hallmarks of AMD is the appearance of drusen both peripherally and in the macula. As there is no conclusive data on Zinc in Drusen, our present study concentrated on demonstrating the presence of readily available or chelatable zinc in drusen in human donor eyes with AMD.

**Methods** Frozen donor eyes from the Montana Eye Bank were used and were photographed before dissection. Using a trephine, 6 mm diameter regions of the peripheral and central retinae were dissected. Following defrosting, the neuronal retina and the retinal pigment epithelial cells were removed to expose the underlying Bruch's membrane with or without drusen. Paired samples from individual eyes were treated in two ways. One of the samples was immersed in phosphate buffer saline (PBS) that contained 100 µM of the zinc specific chelator TPEN and the second sample was in PBS only overnight before 10 µM ZP1 (a zinc specific fluorescence probe) in PBS was applied to both samples for 5 min. Excess ZP1 was removed by rinsing the samples in fresh PBS and the changes in fluorescence was viewed by a NIKON fluorescence microscope (excitation: 460-500 nm; emission: 530-560 nm).

**Results** The samples that were treated with PBS only there was a robust increase in fluorescence in the presence of ZP1, but in the TPEN treated samples the increase in fluorescence was not detectable, signifying the presence of chelatable zinc. Long incubation with ZP1 resulted in punctate staining within drusen that appears to be located within caveoli and certain internal structures in some drusen.

**Conclusion** Therefore, chelatable zinc is present in drusen both in the periphery and in the macula and may play a role in the pathogenesis of AMD.

■ 4141

## Multimodal macula mapping: study for rigid, perspective and deformable image registration.

*BAPTISTA P (1), BERNARDES R (1), CUNHA-VAZ J (1, 2, 3)*
*(1) AIBILI - Association for Biomedical Research and Innovation on Light and Image, Coimbra*
*(2) Center of Ophthalmology, Coimbra University Hospital, Coimbra*
*(3) Faculty of Medicine, University of Coimbra, Coimbra*

**Purpose** The aim of this work is to assess the need for deformable image registration when using SLO based imaging modalities.

**Methods** The image modalities herewith considered are digital RGB color fundus photographs of 50º FOV and 768x576 pixels and retinal leakage analyzer (RLA) images covering the 20º FOV centered on the macula with a resolution limited to 256×256 pixels. The fovea, the major feature common to both modalities, is extracted by a cross-correlation process using a mathematical model of the fovea for each modality, thus providing an initial estimate for the translational part of the rigid registration. Vessel centerlines represented in polar coordinates allows the detection of both the rotation and the foveal displacement (rigid registration).Using the previous result as an initial estimate and vessel bifurcations as landmarks a transformation matrix is computed to produce the perspective registration.For the deformable registration purposes a block search procedure is computed using the partitioned information uniformity. In order to produce the entire mapping, 2D spline surfaces are fitted to the computed reference points.

**Results** The rigid registration is a reasonable approximation. The use of a projective registration in some cases improves the results, but in other examples, where the distribution of the vessel intersection points is not uniform, the algorithm's performance is poor. Deformable registration gives the best results, correcting the local deformations.

**Conclusion** From the different registration methods, the deformable registration is the only one that allows correcting for saccadic movements of the eye when imaged with a raster-scan system.

■ 4142

## Artefacts in Optical Coherence tomography (OCT) imaging of the retina

*STRAUSS RW, THIEL MJ, ULBIG MW, KAMPIK A, NEUBAUER AS*
*Ophthalmology, Ludwig-Maximilians-University, Munich*

**Purpose** To investigate artefacts of OCT-scans and software analysis for retinal cross section scans in an ophthalmological clinic setting.

**Methods** A total of 180 vertical cross-hair OCT-Scans of retinal OCTs were randomly chosen from the database. There were 33% normal findings, the remaining scans showed different retinal pathologies: macula edema, age-related macular degeneration, macular holes, changes of the vitreoretinal border. The frequency of scanning artefacts was analysed and the retinal thickness of all scans was measured automatically in the fovea and 500 µm from the foveal center, using two different software algorithms (Stratus OCT Viewer V 4.01 and Datamedical OCTview V 3.5). Any software errors were assigned into three categories: none, minor (without influence on measurements) and major errors.

**Results** 10% of all OCT-scans showed scanning artefacts. Those influenced significantly the frequency of software-errors (each p=0.012), as did the categorized diagnosis (p=0.004 for Stratus and p=0.01 for Datamedical). Software analysis showed 13% major and 1% minor error for the Stratus OCT viewer and 16% major and 29% minor errors for the Datamedical software (p<0.001). Retinal thickness of both programs correlated very well: r=0.825 for foveal thickness and r=0.867 for parafoveal measurements. Due to a systematic error in the Zeiss algorithm's recognition of the retinal pigment epithelium layer the retinal thickness using Datamedical is approximately 30 µm higher.

**Conclusion** Scanning artefacts were associated with more frequent software errors. Both software programs correlate well, with the Datamedical viewer being more susceptible for minor artefacts. However, in contrast to the Stratus viewer manual corrections are easily possible.

■ 4143

## OCT in patients with X-linked Retinoschisis

*MENKE MN (1), NAKAMURA H (2), HIROSE T (2)*
*(1) Department of Ophthalmology, University of Leipzig, Leipzig*
*(2) Schepens Retina Associates Foundation, Boston*

**Purpose** This study used OCT together with ophthalmoscopical findings to investigate retinal thickness together with a broad spectrum of structural changes in the macula and peripheral retina in patients with X-linked retinoschisis (XLRS) to correlate them with visual acuity (VA) and age.

**Methods** Twenty eyes of 13 males (age range: 3 – 68 yrs) were studied. All patients received VA testing, indirect ophthalmoscopy, slitlamp biomicroscopy, and fundus photography. All patients received OCT scans centered on the fovea. In addition, 8 patients received OCT scans in the peripheral retina.

**Results** VA ranged from 20/40 to 20/400. Retinal thickness was highly correlated with age, indicating retinal elevation in younger patients and progressive atrophy in older patients (p-values ranged from <0.0001 to 0.0013). We found a significant decrease in the frequency of intraretinal cysts (p<0.0001) and tissue pillars (p<0.0181) with age. Multiple other accompanied ophthalmoscopic findings showed no significant correlation with age and VA. Peripheral OCT scans showed cystic retinoschisis in 7 cases and normal retinal appearance in only one case. Previous ophthalmoscopic examination of the particular areas showed no signs of retinal elevation or retinoschisis. Therefore only 12.5% of the ophthalmoscopical findings were in agreement with peripheral OCT findings.

**Conclusion** Younger patients show cystic elevation in several retinal layers, whereas older patients show collapsed retinoschisis with progressed atrophy in OCT images. These morphological changes are highly correlated with age but not correlated with visual acuity.

■ 4144

## Retinal thickness measurements: RTA II vs Stratus OCT in healthy subjects

*NUNES S (1), BERNARDES R (1), BAPTISTA P (1), CUNHA-VAZ J (1,2)*
*(1) AIBILI - Association for Innovation and Biomedical Research on Light and Image, Coimbra*
*(2) Center of Ophthalmology, University Hospital, Institute of Biomedical Research on Light and Image, Faculty of Medicine, University of Coimbra, Coimbra*

**Purpose** To compare retinal thickness in eyes of healthy volunteers using the Retinal Thickness Analyzer (RTAII, Talia Technology, Israel) and the Optical Coherence Tomography (Stratus OCT, Zeiss-Humphrey, USA).

**Methods** Thirty-four eyes from 20 healthy volunteers (14 females/6 males), aged from 40 to 60 years-old (51.5±5.5), were examined in the same session using the RTAII and the Stratus OCT. Using a proprietary software, a new retinal thickness map based on the RTA II data was developed to produce Stratus OCT-like maps (9 areas of: 1mm in diameter central disc area and 8 retinal quadrants -papillo-macular, superior, temporal and inferior- between 1 and 3mm radii and between 3 and 6mm radii). For both maps (RTA and OCT) the mean, SD and 95%CI of the mean were computed for each of the 9 areas. Absolute agreement was considered when both techniques showed the same behavior (over, below or within the 95%CI of the mean). Absolute disagreement was considered when both techniques showed opposite behavior (over/below or below/over).

**Results** All areas for both instruments presented a normal distribution and a statistical significant difference was found between them (p<0.001). The Stratus OCT presents retinal thickness values higher than the RTAII for all areas (21% to 34% higher). Absolute agreement was found in 56% of the cases (areas/eyes) while 8% of the cases showed absolute disagreement. As expected, good agreement was found for the central area (k=0.686,p<0.001) and moderate to poor agreement was found for the remaining areas.

**Conclusion** The Stratus OCT presents retinal thickness values over the RTAII for all of the 9 areas and the best agreement was found to be in central area where Stratus OCT has higher density of retinal thickness measurements.

■ 4145

## Implementing a Colour-Coded Scheme for Earmarking Retinal Changes in a Sequence of Digital Colour Fundus Photographs

*FERREIRA J (1, 2), BERNARDES R (1), BAPTISTA P (1), CUNHA-VAZ J (3, 4)*
*(1) CNTM, AIBILI - Association for Innovation and Biomedical Research on Light and Image, Coimbra*
*(2) Institute of Systems and Robotics (ISR), Coimbra Pole, University of Coimbra, Coimbra*
*(3) AIBILI - Association for Innovation and Biomedical Research on Light and Image, Coimbra*
*(4) Center of Ophthalmology, University Hospital, Institute of Biomedical Research on Light and Image (IBILI), Faculty of Medicine, University of Coimbra, Coimbra*

**Purpose** To assist graders in detecting changes on digital color photographic follow-up time-sequences of the human eye fundus.

**Methods** Sequences of 5 digital color fundus photographs taken every 6 months were assembled from several subjects. All images were normalized and the retinal vascular tree segmented. Vessel bifurcations were then used as landmarks for image registration. Following registration, the differences between each image and the baseline image were computed – each difference-image was considered as a band of a hyperspectral image. PCA was performed, and the most significant principal components were then combined, scaled and transformed into gray-scale images, which were in turn processed so that the contribution of each spectral component (each difference-image) was assigned a specific coloration.A color-coded scheme has been developed which: keeps the earliest earmarking color of areas which have been detected in multiple instances in the follow-up timeline; earmarks areas which have suffered changes but have returned to their original appearance with a specific color-code.Finally, the resulting image is projected onto the baseline image, guiding the grader's attention to changes detected on the fundus photographs.

**Results** Using PCA, the greatest variance of the difference data comes to lie on the primary axes of projection, effectively showing the location of the most significant differences detected throughout the follow-up study.

**Conclusion** Changes detected in retinographic color images were successfully earmarked and their visualization improved using the new temporal color-coded scheme, driving the grader's attention to where meaningful retinal changes might be present.

■ 4146

## Non-invasive Real-time Retinal Temperature Determination during Laser Treatments

*BRINKMANN R (1), KANDULLA J (1), ELSNER H (2), ROIDER J (2), BIRNGRUBER R (1)*
*(1) Medical Laser Center, Lübeck*
*(2) University Eye Clinic, Kiel*

**Purpose** In almost all retinal laser treatments, especially in transpupillary thermo therapy (TTT), the laser induced temperature increase is crucial. Calculations showed that variations in pigmentation, perfusion and pathomorphology can lead to strong temperature differences despite using the same irradiation settings. We investigated a non-invasive optoacoustic (OA) method to determine non-invasively the temperature increase during laser treatments at the fundus, exemplary for TTT.

**Methods** TTT was performed on porcine eye globes in vitro and on rabbits in vivo with a 810nm diode laser for 60s. Additionally, short (1ns) low energy (< 10µJ) Nd:YAG-probe laser pulses (532nm) were repetitively applied for OA excitation. The amplitude of the ultrasonic wave emitted from the fundus depends on the temperature of the absorber, mainly the RPE. For its detection, a standard contact lens was modified with a transducer. Temperature measurements were simultaneously performed with a thin thermocouple.

**Results** The OA determined temperature increase in vitro was proportional to the irradiation, the slope correlates with the thermocouple measurements within +/- 1.2 K. A maximum central spot temperature increase of 8.5 K/100mW for a TTT spot size of 2 mm in diameter was observed. Strong and light pigmented eyes showed a temperature difference by a factor of 2. In rabbits the retinal temperatures at different spots varied by more than a factor of 2 under the same irradiation.

**Conclusion** The optoacoustic on-line temperature determination seems to be an appropriate method for real-time temperature monitoring during TTT. It might serve as a dosimetry control and feedback temperature regulation in TTT and other retinal treatments like photocoagulation.

## ▪ 4151
## Biopsy Of Intraocular Tumors

*ALIGSBURGER JJ*
*Dept of Ophthalmology, University of Cincinnati College of Medicine, Cincinnati, OH*

**Purpose** There is currently no consensus about appropriate indications and contraindications for attempted biopsy of solid intraocular tumors. For purposes of discussion, the author will suggest three general indications for biopsy, one general contraindication to biopsy, and four specific indications for intraocular tumor biopsy. The three suggested general indications for biopsy, all of which should be fulfilled in any given case, are (1) substantial probability that the mass or lesion is a malignant neoplasm or inflammatory tumor, (2) substantial probability that pathologic study of the type of specimen one expects to obtain by the planned biopsy will result in a definitive pathologic diagnosis, and (3) different management options for the lesions or disorders in the differential diagnosis. The suggested general contraindication to biopsy is substantial probability that the biopsy will cause major morbidity that will worsen rather than improve future relevant outcomes for the patient. The four suggested specific indications for intraocular tumor biopsy are (1) major diagnostic uncertainty about the pathologic nature of the mass or lesion of interest – provided that the individual expressing the uncertainty is highly experienced in the field, (2) clinically diagnosed metastatic cancer to the eye in a patient without a prior history of a non-ophthalmic cancer capable of metastasizing or any concurrent pathologically evaluable or antecedent pathologically confirmed metastases in other tissues or organs, (3) clinically diagnosed intraocular lymphoma in a patient with no current evidence or prior history or CNS or systemic lymphoma and minimal or no vitreous cells or a prior inconclusive vitreous biopsy, and (4) request for biopsy by an informed patient who refuses recommended treatment in the absence of pathologic confirmation of the clinical diagnosis. Provided that all three general indications are fulfilled, the general contraindication does not exist, and one of the four specific indications applies, then the ophthalmic surgeon needs to plan a biopsy method that will provide a reasonable balance between the likelihood that the method will yield a representative and sufficient sample of the lesion for definitive pathologic diagnosis and the likelihood that the method will cause problems that worsen relevant patient outcomes. The ophthalmic surgeon also needs to consider the initial and possible future costs of the biopsy method he or she recommends. These considerations will be applied to a discussion of the relative merits of fine-needle aspiration biopsy (FNAB) and alternative methods of intraocular tumor biopsy during this presentation.

## ▪ 4152
## Transvitreoretinal choroidal biopsy of intraocular neoplasms. A + 10 year follow-up of a 10 year consecutive series of biopsied patients

*JENSEN PK (1), SCHERFIG E (2, 1), HEEGAARD S (2), PRAUSE JU (2)*
*(1) Eye Clinic RH2061 Rigshospitalet, Copenhagen*
*(2) Eye Pathology, Copenhagen University, Copenhagen*

**Purpose** To investigate if the survival rate of patients with malignant choroidal melanoma is influenced by performing a transvitreoretinal choroidal biopsy of the tumour during the diagnostic work-up

**Methods** A consecutive series of patients with clinically diagnosed malignant choroidal melanoma, treated at the major tertiary referral centre of Denmark, January 1988 - May 1998, was analyzed. Survival status was obtained from the Danish CPR Registry. Biopsies were grouped according to the predominant cell type in the sample into spindle, mixed, epitheloid and non-specified. Kaplan-Meier survival functions were calculated.

**Results** Patients were treated with enucleation or brachytherapy (Ru-Rh or 125I) according to tumour size and the preference of the patient. 163 persons (91 males and 72 females) accepted to have a transvitreoretinal choroidal biopsy performed. Independent of cell type and treatment 66 ± 4% were alive after five years and 50 ± 4% after 10 years. Of patients with spindle cell type according to biopsy (n=85) 75 ± 5% were alive after five years and 58 ± 6% after 10 years. These results are similar to the survival of patients from the other Nordic countries not having biopsy performed.

**Conclusion** Performing a transvitreoretinal choroidal biopsy of a malignant choroidal melanoma seems not to influence the survival of the treated patients.

## ▪ 4153
## In Vivo Cytogenetic Characterization of Choroidal Melanoma before Brachytherapy

*MIDENA E*
*Department of Ophthalmology, University of Padova, Padova*

**Purpose** Altough uveal melanoma is the commonest intraocular tumor, and thus the subject of much interest for decades, there remain many unanswered questions regarding not only optimal mamagement but also accurate prognostication of metastatic disease. Although less than 2% of the patients have clinically detectable metastases at presentation, hematogenous dissemination probably occurs before the tumor is treated. About 50% of patients with uveal melanoma die because of liver metastases within ten years after tumor diagnosis, irrespective of the type of treatment. Therefore earlier treatment and exact knowledge of prognostic markers are the key factors to decrease mortality from uveal melanoma. The predictive value of classic histological prognostic parameters, such as tumor size, vascular patterns, and cell type have been previously analyzed in detail. Clinical parameters associated with poorer prognosis seem to be extrascleral growth, ciliary body location, and older age. More recently, cytogenetic analysis of uveal melanoma have identified some genetic prognostic markers ( particularly monosomy 3) which are considered more reliable than any other previously considered parameter to identify patients at risk of metastatic disease. Unfortunately cytogenetic characterization was limited to enucleated eyes or surgically resected tumors, leaving most of the conservatively treated patients without any genetic information about their long term prognosis. Recently, the use of cytogenetic techniques has been applied in vivo just before brachytherapy to evaluate some genetic abnormalities of treated tumor. This approach seems to be quite accurate, free of short term complications and safe (on a short term basis).

## ▪ 4154
## Choroidal Tumor Biopsy with 25-gauge Vitrector

*DAMATO B, SEN J, GROENEWALD C, HISCOTT P, SMITH P*
*Royal Liverpool University Hospital, Liverpool*

**Purpose** To describe and evaluate trans-retinal biopsy of choroidal tumors using 25-gauge vitrectomy instrumentation

**Methods** The biopsies were performed under local or general anesthesia, alone or in combination with ruthenium plaque or tantalum marker insertion. Vitrectomy, retinopexy, gas tamponade and suturing of sclerotomies were not usually performed. Histopathology and immunohistochemistry were performed on all samples. Cytogenetic analysis was also performed on melanoma biopsies.

**Results** The surgery was uneventful in all cases and was relatively quick and straightforward. A positive tissue diagnosis was made in almost all patients and cytogenetic studies were also possible, investigating several chromosomes. Inadequate samples led to technical improvements, which have hopefully resolved this problem. Apart from minimal vitreous haemorrhage there were no complications.

**Conclusion** Choroidal tumor biopsy with 25-gauge instrumentation can achieve a sufficient sample for reliable histopathologic diagnosis and, where appropriate, cytogenetic information.

■ **4155**

## Cytologic and Cytogenetic Characteristics of Intraocular Lymphomas

*COUPLAND SE, HUMMEL M, STEIN H*
*General Pathology and Haematopathology Reference Centre, Berlin*

**Purpose** Primary intraocular lymphoma (PIOL) is a high-grade malignant non-Hodgkin lymphoma (NHL) of the retina and vitreous. It can occur togther with a primary central nervous system lymphoma (PCNSL). PIOL is estimated to represent 4-6% of all intracranial tumours and 1-2% of all extranodal NHL. Most PIOL are of B-cell origin (B-PIOL), and can be classified as diffuse large cell B-cell lymphomas (DLBCL).

**Methods** Immunohistochemcially, B-PIOL express B-cell antigens (CD20, CD79a and PAX5), BCL-2, and the immunoglobulin (Ig) transcription factors BOB.1, OCT2a, MUM1/IRF4, BCL-6. Monotypical expression of Ig can usually be demonstrated in PIOL. Expression patterns of Ig transcription factors and Ig vary between PIOL and peripheral DLBCL despite morphological similarities.

**Results** Using IgH-PCR, GeneScan analysis and sequencing, we demonstrated a) a very high mean frequency of somatic mutations detected for the IgH gene in PIOL (14.5%); b) a preferential usage of the VH4-34 gene segment in PIOL; and c) identical clone derivation in oculocerebral lymphoma. Other groups have reported t(14;18) translocation in 54% of B-PIOL. Recently, determination of CDR3 polymorphisms in B-PIOL were described.

**Conclusion** The data suggest that PIOL are a) derived from mature B-cells which have undergone the germinal center reaction, and b) closely genetically related to PCNSL. The latter is underlined by demonstration of the identical clone derivation in oculocerebral lymphoma. Differing patterns of expression of Ig transcription factors and of Ig in PIOL/PCNSL compared to systemic DLBCL supports the notion that peripheral and centrally located DLBCL differ in clinical, immunophenotypic and genotypic features, despite morphological similarities.

■ 4161

## Finding Opportunities for Collaboration Between Researchers in the US and Other Countries

*ATHERTON S*
*Cellular Biology and Anatomy, Medical College of Georgia, Augusta, GA*

**Purpose** To discuss types of collaborations, approaches for finding opportunities for collaboration, and points to keep in mind when entering a collaboration.

**Results** The two most common ways to collaborate with researchers in the United States are long distance collaborations in which the collaborators remain at their respective institutions and collaborations in which the collaborating parties meet and work in one laboratory. Most collaborations result from reading journal articles followed by contact with the author(s), through meeting presentations or seminars resulting in face-to-face discussions about projects, or less frequently, from requests for applications from individuals to conduct research on specific topics as a visiting professor. Collaborations may also result from continuing the relationship between a mentor and a former trainee. Irrespective of the collaboration, the key to building a collaborative relationship is to contact the individual with whom you wish to collaborate, keeping in mind that almost everyone likes to have the opportunity to expand the scope of his/her research via collaboration. The relationship in a collaboration must be mutually beneficial and should not be parasitic, and in any collaboration, it is always wise to discuss the details of who will be responsible for what parts of the project and who will be responsible for writing up the study results in advance.

**Conclusion** With almost instant communication anywhere in the world, there are no intellectual boundaries to collaboration. The only limiting factors in building collaborations may be your ability to maintain a long distance relationship and your patience in dealing/working through inter-country regulatory details or other unanticipated obstacles.

■ 4162

## Funding Mechanisms for Collaboration Between Researchers in the US and Other Countries

*McNICOL LA*

**ABSTRACT NOT PROVIDED**

■ 4163

## Navigating Problems and Pitfalls of Collaborating with US Researchers

*KAUFMAN P*
*Ophthalmology and Visual Sciences, Madison, WI*

**Purpose** To highlight some possible problems that may be encountered in collaborating with US researchers, and their solutions.

**Results** Some areas for discussion will include the following. Permits required for tissue export. Biological safety protocols in place to receive biological agents. Material transfer and research agreements. Subcontracts between US university or with foreign institution. Immigration issues for trainees. Power to assure work gets done diminishes from a distance: sight/out of mind issues; importance of face time, lab – to –lab interactions below the PI level; how to move things along; arranging time together.

**Conclusion** None of the problems mentioned are show stoppers. Collaborations with US researchers are viable options for maximizing resources.

## ▪ 4211
## AlphaCor Outcomes Update

*HICKS CR*
*Biomaterials Research Centre, Lions Eye Institute, Nedlands*

**Purpose** To review current outcome data for AlphaCor.

**Methods** AlphaCor is an approved hydrogel synthetic cornea used for corneal replacement in those judged too high risk for PK. It is implanted within a lamellar pocket opened centrally to the anterior chamber, and opened anteriorly during a second procedure after the skirt region has biointegrated. Pre-operative and post-operative data are prospectively collected for analysis.

**Results** 244 AlphaCor devices were implanted worldwide (to 31/04/05), 46 during formal clinical trials and 198 post-trial. Concurrent systemic conditions were prevalent and prior glaucoma common (54%). Eyes had had 0-13 previous failed grafts (mean 2.4) and original pathologies included trauma/burn (25%), aniridia (6%) and bacterial/viral/fungal keratitis (21%). Follow-up ranged from 1-78 months, mean 13 months. The probability of 1-year retention was 82% in protocol cases. Complications included re-growth of the posterior lamella, stromal melts, and optic deposits. There was a mean gain in over 2 lines of visual acuity with post-op BCVA ranging from PL-6/6.

**Conclusion** Good outcomes can be obtained in cases where PK is not an option. Risk factors for complications have been identified and trends are encouraging. Long term data must continue to be collected for better understanding of the benefits and limitations of this technology.

## ▪ 4212
## Theoretical considerations for glaucoma tubes in KPro eyes

*BARTON K*
*Glaucoma Service Director, Moorfields Eye Hospital, London*

**Purpose** Aqueous shunt devices are often used to manage secondary glaucoma in eyes implanted with a keratoprosthesis. There are a number of such devices available. There are also certain features of keratoprosthesis-implanted eyes that require adjustment in aqueous shunt implantation technique.In this talk, the instructor will describe the key features of aqueous shunt devices that are commonly used, the basic principles of implantation, and adjustments in technique that may be required to accommodate existing or future keratoprostheses.

## ▪ 4213
## Role of mechanical stimulation in corneal tissue engineering

*THEN K (1, 2)*
*(1) Birmingham and Midland Eye Centre, Birmingham*
*(2) Keele University, Stoke-on-Trent*

**Purpose** To examine the role of mechanical stimulation on cellular matrix production and its role in corneal tissue engineering.

**Methods** The application of cyclic mechanical stimulation in bone tissue engineering has been well recognised. Little is known on the role of mechanical strain on corneal keratocytes. We subjected keratocytes grown on 2D surface to cyclic mechanical strain and determine its effect on matrix production and gene expression. We further translate our work in building a novel 3D bioreactor in order to study the effect of mechanical stimulation on the mechanical strength of cell-seeded 'artifical cornea' matrix. It also provided us a new way in studying the effect of environmental cues on corneal mechanical strength.

**Results** Mechanical stimulation on corneal keratocytes increases extracellular matrix production. Gene expression study showed that this is achieved reducing the rate of degradation as oppose to a true increase in collagen production. We also showed that collagen gel seeded with keratocytes exhibit better mechanical strength compared to unseeded collagen gel.

**Conclusion** Mechanical stimulation increases the production of extracellular matrix and mechanical strength of bioengineered corneas

## ▪ 4214
## Analysis of corneal epithelial cell adhesion & growth on poly (methyl methacrylate) PMMA after atmospheric plasma surface treatment

*MOORE JE (1), SMALL DM (2), MEENAN BJ (3), LIU C (3), DOWNES CS (2), MOORE CBT (2)*
*(1) Department of Ophthalmology, RVH, Belfast*
*(2) School of Biomedical Sciences University of Ulster, Coleraine*
*(3) NIBEC University of Ulster, Jordanstown*

**Methods** Surface processing of PMMA was carried out in air at atmospheric pressure using a dielectric barrier discharge (DBD) system. The effect of DBD on surface chemistry was assessed using X-ray photoelectron Spectroscopy (XPS). Primary human corneal epithelial cell adhesion & growth were assessed on modified PMMA. Statistical analysis was used to assess differences in cell growth and proliferation between replicates of modified and unmodified surfaces.

**Results** Three DBD operating parameters namely plasma power (W), processing speed (ms-1) and the number of cycles were investigated resulting in 9 PMMA modifications.XPS analyses assessed the type and scale of the surface chemistry changes which occurred in the top 50 Angstroms. Surface changes varied with surface geographical region. Similarly cellular growth and morphology was also variable. Mean corneal epithelial cell migration and growth was uniformly greater on all modified PMMA surfaces compared to that on unmodified (n=6) this did not reach significance (95% CI) as assessed via a 1-way ANOVA. Inconsistency in cell morphology and growth on the variant modified surfaces suggested that cell interaction with surface depends on local chemical states of carbon. The obvious differences may be consistent with inhomogeneity of plasma discharge. Lack of a statistical significant increase may simply indicate lack of homogeneous DBD effect across treated PMMA.

**Conclusion** Atmospheric plasma surface treatment does appear to increase corneal epithelial cell growth and migration on PMMA but both DBD effect and cellular response vary across surface. More investigations are required to assess & relate chemical modification to cellular response.

■ 4215

## Smart materials for corneal replacements: natural, synthetic and hybrid biomaterials as instructive extracellular microenvironments

*GRIFFITH M (1, 2), LI F (1), FARAJI H (1), BUECKERT D (1), LIU Y (3)*
*(1) University of Ottawa Eye Institute, Ottawa*
*(2) Dept. of Cellular and Molecular Medicine, Univ. of Ottawa, Ottawa*
*(3) BioConstrux Inc., Ottawa*

**Purpose** To tissue engineer a range of "smart materials", i.e. materials that mimic the role of the natural extracellular matrix (ECM) in providing instructive cues to cells during development of organs, or during repair and regeneration. Such materials are being tested as corneal substitutes.

**Methods** Corneal matrix substitutes were fabricated from extracellular matrix molecules such as collagen and reinforced with synthesized polymers. In high risk patients, where extracted biological materials may cause undesirable immune reaction or rejection, recombinant human collagen and completely synthetic biomimetic molecules are used to fabricate corneal matrix substitutes. These bio-synthetic synthetic corneas were tested for biocompatibility in vitro, and also material stability by sub-cutaneous implantation into rats. The best performers were also implanted into rabbits and pigs by lamellar keratoplasty (LKP). Clinical examination (including in vivo confocal microscopy, topography, aesthesiometry) and histopathology was performed.

**Results** Materials developed showed good biocompatibility in vitro, with excellent cell coverage. They also showed stability as sub-cutaneous implants in rats - several were recovered intact. When corneal replacement that were tissue engineered from both extracted and recombinant collagen were implanted into animal models by LKP, they performed well, showing very good integration with the host tissue, with no immune or inflammatory reaction.

**Conclusion** ECM mimics from several sources have the potential to function as tissue engineered corneal constructs, and can be useful for corneal transplantation as alternatives to donor tissues.

■ 4216

## Selection and in vitro Evaluation of a Synthetic OOKP Support Frame

*TIGHE BJ, LLOYD AW, GREEN D, VIITALA R, FRANKLIN VJ, LIU C*
*School of Engineering and Applied Science, Aston University, Birmingham*

**Purpose** OOKP (osteo-odonto-keratoprosthesis) is a complex two-stage surgical procedure requiring the preliminary harvesting of a significant section of tooth and jawbone. We are engaged in the development of a synthetic or semi-synthetic osteo-odonto lamina analogue that could be used in place of the harvested lamina, thus revolutionising OOKP surgery, thereby offering the opportunity to widen the use of the procedure to other patient groups and reducing the cost. An important feature of the target structure is a predictable and minimal rate of bioresorbtion.

**Methods** Our initial approach has focused upon using skeletal frameworks taken from nature, which possess similar structural features to bone and dentine. We also include entirely synthetic bioceramic systems, e.g. titania and silica, as scaffold matrices and coatings.

**Results** Natural porous solids were selected according to pores size, pore interconnectedness, material type and availability. Many objects are found in nature with pores suitable for supporting tissue infiltration and structural support, ranging from protective and ambulatory sea urchin spine to the hard skeletons of Scleractinian corals. Bone-like corals are abundant and easily obtainable. They include proprietary products used in orthopaedic applications, such as Biocoral (calcium carbonate based) and Pro-Osteon (hydroxyapatite based). Other structurally similar materials (e.g.Osteopore & Osteobon) are available and provide a range of potential materials of different chemistries and origins.

**Conclusion** A comprehensive programme is in place to evaluate scientifically the appropriate physicochemical and biological characteristics of modified natural/synthetic materials and thereby identify their equivalence with the dental lamina used in current OOKP surgery., and predict their long term stability. The programme includes procedures for the fabrication and assembly of the composite cornea prototype from its component parts.

## 4221
### Autoregulation and glaucoma

*GUGLETA K*
*University Eye Clinic Basel, Basel*

**Purpose** SIS Lecture.

**Methods** Literature search.

**Results** The functional as well as morphological integrity of the optic nerve head tissue depends on an availability of vital metabolic nutrients and oxygen, which is provided by an adequate perfusion level. Constant blood flow is ensured by autoregulation, a physiologic phenomenon in which the vascular resistance changes dynamically to keep the flow at the level required by the local metabolic activity despite changes in perfusion pressure. Ocular autoregulatory capacity has been investigated in humans using a variety of direct and indirect blood flow estimation techniques. Perfusion pressure was manipulated on the blood pressure side by a posture change, by sudden deflating the thigh cuff or by an isometric exercise. Intraocular pressure has been acutely changed by means of a suction cup, or chronically by therapeutical IOP lowering. Alterations in local metabolic needs were achieved by flicker stimulation, remaining local vascular dilatory capacity was investigated during carbon-dioxide inhalation or after medications. Most of these studies demonstrated a faulty ocular autoregulation in glaucoma patients. In contrast to healthy controls, glaucoma patients show linear correlation between the perfusion pressure and the ocular blood flow. The origin of this autoregulatory disturbance is yet unknown, it should likely be viewed in the context of general vascular dysregulation. Autoregulation in other systems, such as cardiovascular and cerebrovascular, was also found to be defective in glaucoma patients.

**Conclusion** Investigative and therapeutical efforts should therefore rather be directed at restoring the regulatory mechanisms than at simply increasing local circulation per se.

## 4222
### Intraocular pressure fluctuations and variability of blood pressure and heart rate

*NEVES C, BETTENCOURT MJ, POSTOLACHE G, SILVA-CARVALHO L, ROCHA I*
*Physiology, Lisbon*

**Purpose** Before we showed the existence of three bands in the IOP spectrum, similar to those seen in the heart rate (HR) and blood pressure (BP) spectra suggesting an autonomic influence on IOP and the putative clinical application of this method. In this study we investigate the short-term dynamics and patterns of coherence between IOP fluctuations and BP and HR variabilities.

**Methods** Experiments were made on rats, anaesthetized, paralyzed and artificially ventilated. BP, ECG and IOP were monitored and acquired (2kHz). A cross-spectral analysis of BP and RR interval values against IOP fluctuations was performed on 2 minutes baseline condition and 2 minutes after eye atropine administration.

**Results** Systolic BP and RR signals showed a high coherence at low (LF) and high frequency (HF) bands corresponding with sympathetic system and respiratory drive. At these frequencies a positive phase spectra implies that interval variation lead to a pressure variation. A cross-spectrum between BP and IOP signals showed that IOP fluctuations are produced before BP variation at frequencies below 0.2 Hz. For frequencies of 0.2-0.4 Hz and respiratory frequency are induced by BP oscillations. Variations on beat-to-beat intervals lead those of IOP both on LF and HF band suggesting a higher relationship between IOP fluctuations and heart rate variability (HRV). After atropine administration the reverse holds - BP variation leads IOP fluctuations. Also a decreased transfer function between IOP and both BP and HR signal, was recorded to eye atropine administration

**Conclusion** A close link between HRV and IOP was established by cross-spectral analysis This approach seems to offer a new powerful tool for analysis of autonomic involvement on IOP.

## 4223
### Blood flow alterations in glaucoma

*HARRIS A (1, 2)*
*(1) Department of Ophthalmology, Indianapolis, IN*
*(2) Department of Physiology and Biophysics, Indianapolis, IN*

**Purpose** Purpose: To review currently available data on blood flow alterations in primary open angle glaucoma (OAG)

**Methods** Methods: A literature search in May 2005, consisting of a textwordsearch in MEDLINE for the years 1968–2005.

**Results** Results: In the past two decades ocular hemodynamics (OH) assessment has evolved from a subjective description of visible vessels to direct quantitative measurement of blood flow parameters. Each technique examines a different vascular location, indicating the necessity for comprehensive evaluation using various technologies. Commonly used techniques include: color Doppler imaging, ultrasound that measures retrobulbar blood flow velocities; pulsatile ocular blood flow, which determines the pulsatile component of arterial inflow to the eye tonographically from the intraocular pressure pulse (IOP); Heidelberg retina flowmetry, a confocal scanning laser that maps the blood flow in retinal capillaries in arbitrary units; and, fluorescein and indocyanine green angiography using the scanning laser ophthalmoscope, which provides quantitative regional hemodynamic assessment in the retina and choroid. Many studies utilizing different techniques found OH deficits in OAG patients compared to controls. Also, it was recently found that low end-diastolic velocity combined with high resistance to flow in the ophthalmic artery are risk factors for glaucomatous visual field damage over a 7-year followup.

**Conclusion** Conclusions: While IOP reduction remains the primary goal of OAG therapy, there is increasing evidence that other factors, including vascular factors leading to ischemia, may contribute to disease initiation or progression. At present, however, we are lacking longitudinal, prospective, and randomized trials regarding the effect of ocular blood flow modulating on OAG prognosis.

## 4224
### Oxymetry of the optic nerve head

*STEFANSSON E (1), LACOUR M (2), JENSEN P K (2), PEDERSEN D B (2), KIILGAARD JF (2), HOVE M (2), BENEDIKTSSON JA (3), HALLDORSSON GH (3), KARLSSON RA (3), HARDARSON SH (1), BEACH JM (4), EYSTEINSSON T (1, 5)*
*(1) Ophthalmology, Reykjavik*
*(2) Ophthalmology, Copenhagen*
*(3) Engineering, Reykjavik*
*(4) Stennis Space Center, Mississippi*
*(5) Physiology, Reykjavik*

**Purpose** Optic nerve and retinal oxygen tension (ONPO2) reflects the metabolic state of the tissue in ischemic diseases, such as glaucoma, diabetic retinopathy and retinal vein occlusions. We study the effect of intraocular pressure (IOP) on ONPO2 and how ONPO2 is influenced by glaucoma drugs.

**Methods** ONPO2 is measured with polarographic electrodes in pigs and with non-invasive spectrophotometric oximetry in humans. IOP is varied and ONPO2 measured continuously. We studied the effect of glaucoma drugs, especially carbonic anhydrase inhibitors, as well as CO2 and indomethacine.Software development and automatization of spectrophotometric retinal oximetry reduces the variabilty in this technique and makes it easier to perform. We apply this technique to humans in order to test earlier results from laboratory animals.

**Results** ONPO2 is influenced by IOP. With IOP 0-30 mmHg the ONPO2 is stable due to the autoregulation in healthy animals. With IOP above 40 mmHg the ONPO2 decreases linearly with increasing IOP, as the autoregulation is overwhelmed. Lowering the IOP again improves the oxygen tension.Carbonic anhydrase inhibitors, such as acetazolamide and dorzolamide, increase ONPO2 through vasodilatation, which is induced by local hypercapnia and inhibited by indomethacine.

**Conclusion** Optic nerve oxygenation is influenced by intraocular pressure. Lowering IOP by any means improves ONPO2. Carbonic anhydrase inhibitors improve ONPO2 by IOP reduction and also through vasodilation. Human studies with automatic non-invasive retinal oximetry are under way.

■ **4225**

## Drug-induced ocular blood flow alterations

*RECHTMAN E*
*Carmel Medical Center, Haifa*

**Purpose** To review the current available data on the effects of antiglaucoma drugs and systemic medications on ocular blood flow.

**Methods** A literature search in May 2005, which consisted of a textwordsearch in MEDLINE for the years 1968–2005.

**Results** The ocular hemodynamic (OH) effect of the majority of the commonly used anti-glaucoma medications has been studied using a variety of techniques. Several systemic medications, including calcium-channel blockers and ACE inhibitors have also been evaluated for their OH effects. Based on the current data, there is evidence that a few antiglaucoma medications have the potential to improve ocular blood flow. These mainly include the systemic and topical carbonic anhydrase inhibitors. Additionally, the fixed combination of timolol and dorzolamide does not alter the enhancing effect of dorzolamide on retinal hemodynamics. Among the systemic medications tested, calcium channel blockers do not evoke a uniform response in glaucoma patients, while ACE inhibitors were found to elicit no OH effect.

**Conclusion** Many studies utilizing different techniques found OH deficits in primary open angle glaucoma patients compared to controls. There is an urgent need for long-term, prospective, randomized studies to examine the effect of ocular blood flow modulation on OAG prognosis. If such studies were to demonstrate a protective effect against disease progression, a new era in glaucoma treatment would ensue.

■ 4231

## Predictive factors for predominantly classic choroidal neovascular membrane enlargement following photodynamic therapy.

*FLETCHER EC (1), KHAN J (1), MENON G (2), CHONG NHV (1)*
*(1) Kings College Hospital, London*
*(2) Frimley Park Hospital, Surrey*

**Purpose** Enlargement of choroidal neovascularisation (CNV) after photodynamic therapy (PDT) is commonly associated with poor visual outcome. We would like to assess whether there are any predictive features of enlargement on baseline angiogram.

**Methods** Analysis of photographic and angiographic features in 155 patients with predominantly classic CNV in two retinal centres, King's College Hospital, London and Frimley Park Hospital, Surrey. The following characteristics were evaluated: i) the percentage change in size of the CNV lesion 3 months following first PDT ii) amount of leakage of CNV lesion at baseline iii) grading into 100% or predominantly classic iv) presence of associated retinal haemorrhage v) presence of a choroidal halo and/or discrete lesion.

**Results** 97 eyes showed significant CNV enlargement following PDT. 58 eyes were classified as non-enlarging. The mean amount of leakage of the CNV lesion at baseline in the enlarging group (50.74%) was significantly different (p = 0.004) compared to the non-enlarging group (16.60%).

**Conclusion** PC-CNV lesion leakage at baseline is significantly associated with patients who have CNV enlargement following first PDT treatment. We provide an objective way of predicting which patients will have CNV enlargement following PDT. This data may be useful when considering the use of adjunctive therapy such as intravitreal triamcinolone or anti-VEGF therapies. Prospective studies are required to evaluate the accuracy of baseline CNV leakage as a predictive factor of CNV enlargement following PDT.

■ 4232

## Consequences of verteporfin photodynamic therapy on choroidal neovascular membranes

*TATAR O (1), ADAM A (2), SHINODA K (3), VÖLKER M (1), LAFAUT B (4), BARTZ-SCHMIDT KU (1), GRISANTI S (1)*
*(1) Department of Ophthalmology, Eberhard-Karls University, Tuebingen*
*(2) Department of Pathology, Eberhard-Karls University, Tuebingen*
*(3) Department of Ophthalmology, Keio University, Tokyo*
*(4) Department of Ophthalmology, AZ St Jan, Bruges*

**Purpose** To examine the impact of photodynamic therapy (PDT) on angiogenesis in human choroidal neovascularization (CNV) membranes with respect to Vascular Endothelial Growth Factor (VEGF) expression, proliferation and vascularization

**Methods** Retrospective review of interventional case series of fifty patients (fifty eyes) who underwent removal of CNV. CNV was secondary to age-related macular degeneration (AMD) in all cases. Twenty of these patients were treated with PDT 3 to 655 days before surgery. CNV were stained for CD34, CD105, Ki-67, cytokeratin18 and VEGF. Thirty CNV secondary to AMD without previous treatment were used as control.

**Results** Specimens without pre-treatment disclose varying degrees of vascularization, proliferative activity and VEGF expression by different cells. Specimens that had been treated by PDT three days previously show mostly occluded vessels, damaged endothelial cells and low proliferative activity. In contrast, specimens excised at later time points after PDT were highly vascularized and proliferating. This chronology is associated with an impressive VEGF immunoreactivity unique to retinal pigment epithelial cells (RPE) shortly after PDT that shifts also to other cells at later time points.

**Conclusion** PDT induces a selective vascular damage in CNV. The effectiveness and selectivity of this treatment, however, seem to be jeopardized by a rebound effect initiated by an enhanced VEGF expression in RPE cells.

■ 4233

## Inter-eye difference in exudative age-related macular degeneration with minimally classic or occult subfoveal neovascularisation after unilateral intravitreal injection of triamcinolone acetonide

*JONAS JB, SPANDAU UH, HARDER B, VOSSMERBAEUMER U, KAMPPETER BA*
*Department of Ophthalmology, Faculty of Clinical Medicine Mannheim of the University Heidelberg, Mannheim*

**Purpose** To report on visual outcome of patients receiving intravitreal triamcinolone acetonide for treatment of exudative age-related macular degeneration with minimally classic or occult subfoveal neovascularisation.

**Methods** The study included 20 consecutive patients with bilateral exudative age-related macular degeneration. An unilateral intravitreal injection of about 20 mg triamcinolone acetonide was performed into the eye (study group) more severely affected or showing more pronounced progression of the disease. The contralateral eyes served as control group. Mean follow-up was 13.5 ± 4.1 months (3.6 – 34.6 months).

**Results** In the study group, visual acuity increased significantly (p<0.001) from 0.96 ± 0.32 logMar to a mean maximum of 0.76 ± 0.30 logMar during follow-up. An increase in best visual acuity during follow-up was found in 18 (90%) eyes. In 11 (55%) eyes and 7 (35%) eyes, respectively, best visual acuity increased by at least two Snellen lines and three Snellen lines, respectively. In the control group, visual acuity at baseline and the highest visual acuity measurements during follow-up did not vary significantly (p=0.90). In 5 (25%) eyes and 2 (10%) eyes, best visual acuity increased by at least two Snellen lines and three Snellen lines, respectively. Comparing study group and control group, visual acuity gain was significantly (p=0.003) higher in the study group.

**Conclusion** Intravitreal triamcinolone acetonide may temporarily increase visual acuity in eyes with exudative age-related macular degeneration.

■ 4234

## Photodynamic therapy and intravitreal triamcinolone at high doses to treat exudative age related macular degeneration: one year outcome.

*MONTERO-MORENO JA, RUIZ-MORENO JM, BARILE S*
*VISSUM, Alicante*

**Purpose** To evaluate the efficacy of photodynamic therapy with verteporfin (PDT) and intravitreous triamcinolone at high doses to treat choroidal neovascularization (CNV) associated to age related macular degeneration (AMD).

**Methods** Prospective, consecutive, comparative, non randomized, interventional case series. Thirty consecutive eyes from 30 patients with subfoveal CNV associated to AMD were treated by PDT followed by the intravitreal injection of 19.4+/-2.1mg /0.1ml triamcinolone five days later. Fifteen eyes had received no previous treatment (Group 1) and fifteen had been previously treated by PDT alone (Group 2). 15 eyes treated by PDT alone, matched for age, CNV composition and size were used as controls. Corrected visual acuity (BCVA) (LogMAR), number of lines improved or lost (Snellen), number of PDT sessions needed to achieve CNV closure and adverse events were considered.

**Results** Mean BCVA at baseline was 0.72+/-0.35 compared to 0.65+/-0.33 at month 12 (LogMAR) (P=0.49) in Group 1; mean BCVA at baseline in Group 2 was 0.74+/-0.32 compared to 0.77+/-0.27 at month 12 (P=0,10). Mean baseline BCVA in the Control Group was 0.80+/-0.33 compared to 1.03+/-0.52 (P=0.04) (Student t test for paired data). Fluorescein angiography demonstrated the presence of leakage in 35.7%, 21.4%, 7.1% and 0% of eyes at months 3, 6, 9 and 12 respectively in Group 1; in 6.6%, 13.3%, 0% and 0% of eyes in Group 2; and in 93%, 66.6%, 46.6% and 33.3% of eyes in the Control Group. Intraocular pressure rose in 8 /14 eyes in Group 1 and in 7/14 eyes in Group 2. Cataracts developed in 8/28 eyes.

**Conclusion** PDT followed by intravitreal injection of triamcinolone may be helpful to treat exudative AMD.

■ **4235**

## Polypoidal choroidal vasculopathy in Korean patients with large submacular hemorrhage

*LEE SC, YOON JS, LEE JH, KOH HJ, KWON OW*
*Department of Ophthalmology, Yonsei University College of Medicine, Seoul, Korea, Seoul*

**Purpose** To determine the incidence of hemorrhagic polypoidal choroidal vasculopathy(HPCV) in Korean patients with submacular hemorrhage, and to compare clinical characteristics, visual prognosis and treatment of HPCV with those of hemorrhagic choroidal neovascularization(HCNV) due to age-related macular degeneration(ARMD).

**Methods** Retrospective analysis of 44 consecutive eyes with a submacular hemorrhage comprising more than 50% of the neovascular lesion. Patients were diagnosed as having HPCV or HCNV on the basis of indocyanine green angiography.

**Results** Of the 44 eyes with submacular hemorrhage, 26 were classified as HPCV and 18 as HCNV. The baseline patient characteristics were similar for both groups. At the final follow-up the HPCV group had 17 eyes showing visual improvement, 4 eyes showing maintained vision, and 5 eyes showing visual deterioration. In contrast, the HCNV group had 4 eyes showing visual improvement, 1 eye showing maintained vision, and 13 eyes showing visual deterioration. Visual acuity < 0.1 was present in 7(27%) HPCV eyes and 10(56%) HCNV eyes. PDT was performed in 15 HPCV eyes, of which 13(87%) showed improvement or no change in visual acuity, while only 2(22%) of the 9 HCNV eyes responded similarly after PDT. Eyes treated with PDT did not have better outcomes compared to eyes that underwent other types of treatment.

**Conclusion** PCV accounts for the largest proportion of submacular hemorrhage in Koreans. PCV showed a better visual prognosis than CNV. Although PDT was effective in obliterating polypoidal lesions and refining visual acuity, it was not found to be superior to other treatment modalities.

■ **4236 / 447**

## Morphological Features of Classic and Occult subfoveal Choroidal Neovascular Membrane using OCT -An aid to diagnosis

*ADEWOYIN TA, KHAN J, CHONG NHV*
*Laser and Retinal Research Unit, King's College Hospital, London*

**Purpose** To assess the morphological differences of choroidal neovascular membrane on optical coherence tomography (OCT) in classic and occult CNV.

**Methods** Consecutive case series of 57 eyes of 57 patients diagnosed wih acute CNV undrwent OCT-3 examination, followed by fluorescein angiogram.Fluorescein angiograms were reported using TAP guidelines.The OCT images were assessed based on the presence of intraretinal fluid and subretinal fluid, the presence of a discrete subretinal lesion and the presence of pigment epithelial detachment.

**Results** Regression analysis suggested that classic lesions are more likley to have a discrete sub retinal lesion and intraretinal fluid, whereas occult CNV are more likely to have fluid in the sub retinal or RPE space. Using these characteristics,96% of classic lesions and 70% of occult lesions had shown the above mentioned respective features.

**Conclusion** Optical coherence tomography is a non invasive powerful tool. Morphological differences between classic and occult neovascular lesions enable their diagnosis using OCT with reasonable accuracy compared to the gold standard of fluorescein angiogram.

■ **4237 / 448**

## Photodynamic therapy versus surgical removal from choroidal neovascularisation in high myopia

*HERA R, VINH V, GONZALVEZ B, MILLET JY, ROMANET JP, MOUILLON MM*
*Ophthalmology, Grenoble*

**Purpose** Several treatments of choroidal neovascularization (CNV) in pathologic myopia are proposed today. We compared, by a retrospective analysis, the visual outcome at 12 and 24 months between patients who underwent surgical removal of CNV and patients treated by photodynamic therapy(PTD).

**Methods** We reviewed, after two years of follow up, clinical and angiographic findings of 25 patients with severe myopia and subfoveal classic CNV. The patient population was divised in 2 groups:11 patients treated by PTD and 14 patients treated by surgery.

**Results** An intermediary analysis was performed at 12 months and showed a significant visual acuity benefit for the group treated by surgery. The final results at 24 years showed no significant diffference in the visual outcome between the two groups.

**Conclusion** Even if the surgical removal is more benefic for the visual outcome in the first year, the final visual acuity is quite similar for the two groups after two years of follow up. In the light of this results we conclude that in CNV of pathologic myopia, PTD is preferable to surgical removal because is more safe.

## 4241
### Electrophysiologic testing in unexplained visual loss

*LAM BL*
*Bascom Palmer Eye Institute, Miami, FL*

**Purpose** To demonstrate the utility of electrophysiologic testing in patients with unexplained visual loss.

**Methods** Retrospective review and case examples from an active electrophysiologic service and neuro-ophthalmology center.

**Results** During the 10-year period 1995-2004, approximately 40% of new patients seen at our neuro-ophthalmology service were referred with unexplained visual loss or symptoms. Comprehensive history and examination combined with appropriate use of ancillary modalities including electrophysiology testing were critical in identifying underlying etiologies. Common conditions identified by electrophysiologic testing included occult retinal dystrophies, retinal inflammatory conditions, paraneoplastic retinopathies, previous retinal vascular occlusions, localized retinal dysfunction, and subclinical optic neuropathy.

**Conclusion** Electrophysiologic testing should be considered as an integral part of the work-up for patients with unexplained visual loss.

## 4242
### Functional and anatomical imaging of the retina: combining OCT and SLO imaging with multifocal ERG

*KEATING D, DUDGEON S, PARKS S*
*Tennent Institute of Ophthalmology, Glasgow*

**Purpose** To combine multifocal electrodiagnostic functional imaging with anatomical optical coherence tomography imaging and scanning laser ophthalmoscope imaging.

**Methods** A new commercial OCT/SLO scanner provides high-resolution and high definition images of the surface anatomy of the fundus and subsurface tissue structure using a combination of confocal imaging principles of SLO and cross-sectional imaging of OCT. A modification of the syetem to include an organic light emitting diode (OLED) micro display within the optics of the optical coherence tomography system enables simultaneous functional multifocal ERG imaging and OCT /SLO imaging.

**Results** The OLED display can be used to provide micro stimulation of the fundus producing micro functional images of the macula which can be used to complement the powerful anatomical imaging technologies of OCT and SLO.

**Conclusion** The new technique should provide improved resoloution and greater accuracy in the assessment of retinal disorders without compromising the capabilities and flexibility of either technology

## 4243
### Electrophysiology in relation to autofluorescence imaging in retinal dystrophies

*HAWLINA M*
*University Eye Clinic, Ljubljana*

**Purpose** Fundus autofluorescence (AF) shows accumulation of lipofuscin in the retinal pigment epithelium in vivo. The production of lipofuscin is enhanced in early stages of retinal dystrophies and reduced or absent in the late stages. Fundus AF may be recorded either by scanning laser ophthalmoscope or by fundus camera using appropriate filter. The aim of this presentation is to compare the AF images with electrophysiological and microperimetry (MP).

**Methods** In diseases such as RP, cone-rod dystrophy, Stargardt disease, Best macular dystrophy, enhanced s-cone syndrome or XLRS, AF patterns were correlated with electrophysiological tests. AF images were obtained using a confocal scanning laser ophthalmosope (HRA, Heidelberg Retina Angiograph, Germany) or digital fundus camera (TOPCON). Pattern of AF was of both systems was analyzed also in relation to retinal function tested with fundus-controlled microperimetry (MP1, Nidek, Japan).

**Results** Hyperfluorescent areas corresponded to dysfunctional retina whilst areas of low or absent AF depicted atrophic, non-functional retina. Distinct hyperfluorescent rings and other patterns of AF have been seen in different diseases and corresponded well to mfERG and MP. Moreover, AF imaging showed cystic spaces in the macula of ESCS and XLRS patients that were not visible with fluorescein angiography.

**Conclusion** Autofluorescence imaging is both safer and better than fluorescein angiography for diagnosis of retinal dystrophies. mfERG and MP confirmed that AF imaging contains both structural and functional information. The advantage of fundus camera was quicker recording of AF of a greater field of view (central 50 degrees compared to 30 degrees in HRA), however it may have not show some patterns, seen with the HRA.

## 4244
### The effect of light as a stimulus and light as an environment on retinal function

*LACHAPELLE P (1), RUFIANGE M (2), BEAULIEU C (2), DUMONT M (2)*
*(1) Ophthalmology- McGill University/Montréal Children's Hospital, Montréal, Québec*
*(2) Psychiatry, Université de Montréal, Montréal, Québec*

**Purpose** The electroretinogram (ERG) identifies the electrical response generated by the retina. While the relationship between stimulus energy and scotopic (rod) responses is well documented, we examined the impact of the luminous environment on the amplitude and shape of the photopic (cone driven) potential.

**Methods** Photopic (cone mediated) luminance-response functions were generated from normal subjects under different luminous environments. (Short term adaptation): Brief exposures to 17 and 30 and 500 cd.m-2 rod desensitizing backgrounds; (Gradual adaptation): during light adaptation and (Long term adaptation): following at least 4 months of working in a dim or bright luminous environments or following exposure to short or long photoperiods (winter vs summer measurements).

**Results** As expected, for a given intensity of stimulation, the amplitude of the resulting cone-mediated ERG decreased as the intensity of the surrounding luminous environment increased (short term adaptation), causing a rightward shift of the luminance-response function. While the same relationship is obtained following gradual adaptation, the exact reverse is observed following long term adaptation.

**Conclusion** The photopic ERG is a complex response that takes into consideration the immediate luminous environment as well as the short and long term light history to which the retina was subjected prior to testing. While short term adjustments might imply a rapid (neural) adaptation of the retina, long term adjustments might involve a photostasis-like effect as suggested from animal work. This should be kept in mind when recording and interpreting the cone-mediated ERG responses, especially when a retinal disorder is suspected. Supported by CIHR and FRSQ-Vision Network

■ 4245

## Genetic ophthalmology & electrophysiology: inseparable conjoined twins

*LEROY BP (1, 2)*
*(1) Department of Ophthalmology, Ghent University Hospital, Ghent*
*(2) Center for Medical Genetics, Ghent University Hospital, Ghent*

**Purpose** To describe the essential role of visual electrophysiological tests in the genetic ophthalmology clinic.

**Methods** A case presentation format will be used to illustrate the essential role of electrophysiological tests in the genetic eye clinic.

**Results** ISCEV-standard electrophysiological tests play an essential role in making an exact diagnosis in inherited retinal diseases. Moreover, regular follow-up including electrophysiological re-evaluation provides essential information with regard to visual prognosis of the patient. Additionally, both clinical and electrophysiological phenotypes combine in an inseparable way to suggest potential genotypes underlying the condition.

**Conclusion** Both for making an exact diagnosis and for prognostic predictions, visual electrophysiological test are essential in the genetic ophthalmic practice.

■ 4246

## Electrophysiology and inflammatory eye disease

*HOLDER GE*
*Moorfields Eye Hospital, London*

**Purpose** To describe the role of electrophysiological investigation in the diagnosis and mangement of retinal inflammatory disease.

**Methods** The techniques used for recording electrophysiological signals will be described, with particular reference to the use of ISCEV Standard recordings (the International Society for Clinical Electrophysiology of Vision). The application of the results will be discussed using illustrative cases.

**Results** Electrophysiology has different roles in the management of inflammatory disease. Initially, it may confirm the nature and severity of the disease, not always apparent from symptoms or ocular signs, and may be diagnostic in disorders such as AZOOR (acute zonal occult outer retinopathy). Electrophysiology can also provide objective data in relation to the efficacy of treatment, enabling management decisions to be taken with greater confidence. This is particularly important in disorders such as birdshot chorioretinopathy where management is not facilitated by a highly unpredictable clinical course.

**Conclusion** Electrophysiological testing provides objective data to contribute to the mangement of the patient with retinal inflammatory disease.

## ▪ 4252
## Primary Retinal Lymphoma

*BURNIER MN, ODASHIRO AN, MARSHALL JC*
*Henry C Witelson Ocular Pathology Laboratory, Mcgill University, Montreal*

**Purpose** Ocular lymphoma comprises several types of lymphoma, each of them related to different ocular environment, e.g. Eyelid - T cell lymphoma (mycosis fungoides), Conjunctiva - MALT B cell lymphoma, Retina - Primary large B cell Lymphoma, Choroid and Orbit – B cell Lymphoma. Primary intraocular lymphoma of the retinal is a bilateral large B-cell lymphoma with extensive areas of necrosis, more common in female patients and presents as a masquarede syndrome/uveitis, being associated with central nervous system involvement.

**Methods** We studied 20 patients with primary retinal lymphoma. From 7 patients with intraocular and CNS tumor, tissue samples were obtained for flow cytometry and DNA & S-phase fraction.

**Results** Seventeen female and 3 male patients with mean age of 55.2 year-old (range 37-57 year-old), 16 were immune-competent and 4 immune-suppressed. The mean time of onset of symptoms to diagnosis of intraocular lymphoma was 23.6 months (range 1-69 months). Immunohistochemistry was positive for LCA, CD20 and CD26, indicating a B cell tumor. The flow cytometry and DNA & S-fraction samples from the intraocular and CNS indicated a higher proliferation rate (23,8%) for the ocular component.

**Conclusion** Primary retinal lymphoma is a bilateral large B-cell lymphoma that affects female patients and presents as uveitis (masquerade syndrome). The different proliferation rates of intra-ocular and CNS lymphomas may be explained by their different spatial location or a distinct genetic composition, reinforcing the hypothesis that the two are different entities.

## ▪ 4253
## Histopathological findings, prognostic factors and differential diagnosis of lymphoproliferative lesions of the ocular adnexa

*COUPLAND SE*
*General Pathology and Haematopathology Reference Centre, Berlin*

**Purpose** Ocular adnexal lymphoproliferative tumours include reactive lymphoid hyperplasia (RLH) and malignant lymphoma. The ocular adnexal lymphomas (OAL), which are extranodal Non-Hodgkin lymphoma (NHL), can develop as primary or secondary tumours. The extranodal marginal zone B-cell lymphoma (EMZL) represents the most common lymphoma subtype followed by diffuse large cell B-cell lymphomas, follicular lymphomas and mantle cell lymphomas. OAL of non-B-cell type are infrequent, and Hodgkin lymphoma of the ocular adnexa is exceptionally rare.

**Methods** Ocular adnexal EMZL have similar clinical, pathological and molecular features to those EMZL arising in other locations. They are associated with autoimmune diseases, and reportedly with Chlamydia psittaci. Frequent chromosomal abnormalities include t(11;18), t(14;18), t(3;14) and t(1;14). Most patients are diagnosed in Stage IE, and demonstrate an indolent clinical course; systemic dissemination does however occur. Ca. 40% of patients may have Stage II or more disease at diagnosis. A typical feature of recurrent EMZL is involvement of contralateral ocular adnexal tissues and/or other extranodal sites. A "high-grade" transformation of EMZL has been reported.

**Results** Major prognostic criteria for the OAL include anatomic location of the tumour (conjunctival OAL better prognosis); stage of disease at first presentation (Stage IE versus Stage II and above); serum lactate dehydrogenase level at the time of diagnosis (decreased versus increased); lymphoma subtype (EMZL versus non-EMZL); and tumour cell growth rate.

**Conclusion** The clinical and histopathological findings of the differential diagnoses of lymphoproliferative lesions of the ocular adnexa are discussed.

## ▪ 4254
## Conjunctival MALT Lymphomas

*VAN DER VALK P*
*pathology VUMC, Amsterdam*

**Purpose** Conjunctival lymphomas will be presented and discussed here. This is a diverse group of tumours that not infrequently cause confusion, but a background to these tumours will be presented for clarity.The most frequent type is the extranodal marginal zone lymphoma and its morphology, with its characteristic lymphoepithelial lesions and follicular colonization will be presented in a little more detail. Also, the immunophenotype will be put forward, vis-a-vis the differential diagnosis of other lymphoma types and reactive conditions. Other lymphoma types include lymphoplasmacytic and plasmacytic, folicular and diffuse large B-cell lymphomas and their histology and immunophenotype will be presented as well.The background is the Mucosa-Associated Lymphoid Tissue (MALT) of which the conjunctiva forms a part. This immune system features specific lymphocytes that have a set of specific functions and a corresponding morphology and immunology. The cells of tumours arising from this system will retain these functions, morphology and immunology for a large part. Thus, knowledge of the normal situation can significantly enhance insight in this group of tumours and the different lymphomas will be set in this context.

## ▪ 4255
## Lacrimal System Lymphomas

*PRAUSE JU, SJö L, HEEGAARD S*
*Eye Pathology Institute, University of Copenhagen, Copenhagen*

**Purpose** To describe clinical and histopathological aspects of lacrimal gland and tear sac lymphomas

**Methods** All lymphocytic lesions of the lacrimal gland registered in the Dansih Orbital Tumour Database since 1974 were collected. Cases of tear sac lymphomas were collected from eye pathology institutes registered as members of European Organization for Research and Treatment of Cancer or European Ophthalmic Pathology Society during the period 2003-2004.Histopathological analysis of peraffin sections using appropriate panels of immunoglobulins was performed and classification of lymphoma types according to the WHO classification was obtained.Findings were correlated tp published series of lymphomas of the ocular region containing data on lacrimal system lymphomas.

**Results** Thirteen lacrimal gland lymphomas were identified. Extranodal marginal zone B-cell lymphoma (MALT-lymphoma) was most frequent.Fifteen tear sac lymphomas were collected from Europe. Five of these were diffuse large B-cell lymphomas, five were MALT-lymphomas, three could be characterized as being in transition from MALT- to diffues large B-cell lymphoma. Two could onle be described as B-cell lymphomas

**Conclusion** Lymphoma of the lacrimal gland is most frequently MALT-lymphoma like lymphoma in the most of the eye region. Lymphoma of the tear sac is very rare and differs from the other eye region lymphomas being frequently of the diffuse large B-cell type.

■ **4256**

## New Therapeutic Approaches in Ocular and adnexal Lymphomas

*DECALUDIN D*
*Department of Clinical Hematology, Institut Curie, Paris*

**Purpose** Intraocular and ophthalmologic lymphomas constitute two different entities in terms of clinical and pathological features and prognosis. The first, but less frequent group, predominantly composed of high-grade B-cell non-Hodgkin's lymphomas (NHL), can be assimilated to primary brain lymphoma for which management usually comprises methotrexate-based combination chemotherapy and radiotherapy. The second group, defined by intraorbital and/or adnexal lymphoma, presents several specific characteristics: (1) almost all cases were B-cell non-Hodgkin's lymphoma, which was low-grade in about 80% of cases. About 50% of patients in this population had a MALT type NHL, while the other two most representative histopathological subtypes were lymphoplasmocytic and follicular lymphomas; (2) orbital and lacrimal gland sites are the most frequent ophthalmologic sites, with bilateral ophthalmologic involvement in 10% of cases; (3) extra-ophthalmologic disease was observed in about one-third of patients, with nodal involvement in 20% of cases and bone marrow involvement in 10% of cases; (4) the outcome of lymphomas with ophthalmologic involvement at diagnosis was not different from that observed for nodal NHL, namely better survival in low-grade cases without stabilization of the survival curves, and a stabilized OS of 50% for the high-grade group The therapeutic management of ophthalmologic lymphomas must therefore take all of these characteristics into account, namely the pathological subgroup according to the WHO classification, the ophthalmologic site including evaluation of the immediate risk to the eye and its function, and the stage of the disease with tumor burden determination. While high-grade NHL always justify a combination chemotherapy regimen, low-grade lymphomas can be treated by exclusive radiotherapy, radiotherapy plus single-agent or combination chemotherapy, monoclonal anti-CD20 antibody immunotherapy, anti-Chlamydia psittaci antibiotic therapy, or observation and a "wait-and-see" policy. These multiple treatment options emphasize the lack of clear consensus in the treatment of low-grade ophthalmologic lymphomas and the need for multicenter prospective studies.

## ▪ 4261
## Effects of SOCS-1 gene transfer in ARPE cells

*WILLERMAIN F (1, 2), KISMA N (1, 2), DE GREEF S (1, 2), KOCH P (1, 2), CASPERS L (1), BRUYNS C (2)*
*(1) Ophthalmology, CHU St-Pierre, ULB, Brussels*
*(2) IRIBHM, Campus Erasme, ULB, Brussels*

**Purpose** IFNγ is a major cytokine implicated in intraocular inflammation (IOI) development. By inducing suppressor of cytokine signalling (SOCS) protein synthesis, IFNγ signalling possess a negative feed-back loop. Stimulation of such natural immunosuppressive pathway might be a promising approach to treat IOI. Here, we have investigated the effects of SOCS1 over-expression on the activation of ARPE cells by IFNγ.

**Methods** ARPE cells were transfected by a plasmid containing SOCS1 and stimulated by IFNγ. Expression of MHC class I, MHC class II, and CD54 was analysed by flow cytometry. CIITA (Class II Trans-Activator), IRF-1 and IRF-2 (Interferon Regulatory Factors) transcription was assessed by RT-PCR.

**Results** SOCS-1 over-expression in ARPE cells inhibited IFNγ-mediated MHC II induction while CIITA, IRF1 and IRF2 transcription was not affected. Interestingly, SOCS-1 transfection increased MHC I basal expression, and CD54 IFNγ-mediated upregulation.

**Conclusion** SOCS-1 over-expression in ARPE cells exerts a strong inhibitory effect on the IFNγ-induced MHC II expression, but have also positive effects on MHC class I and CD54 expression. Further studies are thus needed to precise the possible role of SOCS1 in modulating IOI.

## ▪ 4262
## CCR2 V64I polymorphism as a genetic risk marker of Behcet's associated uveitis

*AHAD M A (1, 2), MISSOTTEN T (1), LIGHTMAN S (1, 2)*
*(1) Moorfields Eye Hospital, London*
*(2) Institute of Ophthalmology, London*

**Purpose** CC chemokines are important chemo attractants for Th1 cells that play crucial role in Behcet's disease and different form of uveitides as well. Single nucleotide polymorphisms at promoter sites of MCP-1 -2518, RANTES -403, and CCR5 -59029 affect the expression of the respective genes and are found to be associated with various Th1 mediated autoimmune diseases. Similarly 32bp deletion in CCR5 gene and V64I polymorphism in CCR2 gene have been associated with autoimmune diseases. The aim of this study was to determine whether any of these polymorphisms are associated with uveitis and visual outcome in Behcet's disease.

**Methods** 31 Caucasian patients with Behcet's associated uveitis and 126 age and ethnically matched controls were genotyped for MCP-1 -2518A/G, RANTES -403G/A, CCR2 V64I, CCR5 -59029G/A and CCR5 32bp deletion, by single specific primer polymerase chain reaction method. Genotype distribution between the groups was compared by chi square test. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS 12.0 Chicago USA).

**Results** The frequency of CCR2 64I allele was 18% in patients compared to 6% in controls (pc=0.006, OR; 3.08, 95%CI=1.48-6.41). The 64I allele carriage was more common in female (67%) compared to males (18%) pc=0.04. Also the frequency of 64I allele was 38% in patients on second line immunosuppressive therapy, as compared to 14% in patients not requiring second line immunosuppressive treatment (p=0.03).

**Conclusion** CCR2 64I polymorphism can predispose patients to Behcet's associated uveitis and can affect the visual outcome.

## ▪ 4263 / 476
## Serum Prolactin Levels and Behçet Disease

*PROENCA H, GONÇALVES C, FERREIRA C, MIRANDA M, CASTANHEIRA-DINIS A, MONTEIRO GRILLO M*
*Ophthalmolgy, Lisbon*

**Purpose** To evaluate basal prolactinemia in Behçet's Disease (BD) and correlate with phenotypic expression.

**Methods** We compared 22 patients fulfilling BD Research Committee criteria with 21 age- and sex-matched healthy control subjects. Age, clinical manifestations, HLA-phenotyping and therapy were analysed.

**Results** Prolactinemia was significantly higher (mean=19,34 ng/mL) in BD's patients vs. controls (mean=9,83 ng/mL) (p=0,039). This difference was even higher in complete-type BD subgroup vs. controls (p=0,001). Younger patients (<30 y) required corticosteroids plus immunosuppressives more often (80%) suggesting a correlation between age and disease severity.

**Conclusion** Results suggest the role of prolactin in BD pathogenesis and its correlation with disease expression, especially in complete-type BD.

## ▪ 4264
## Fluorophotometry in patients with chronic posterior uveitis treated with intravitreal injections of triamcinolone acetonide

*THOMAS V, PATTE M, COULANGEON LM, BACIN F*
*Ophthalmology, Clermont Ferrand*

**Purpose** To evaluate the effect of triamcinolone acetonide intravitreal injections on the blood retinal barrier permeability by fluorophotometry

**Methods** Fluorophotometry of the vitreous evaluates blood retinal barrier permeability by measuring fluorescein concentration in the vitreous after intravenous injection.6 eyes in 5 patients aged 44 to 78 years were injected one or two times and were compared to non-injected eyes. Observational procedures included: visual acuity, biomicroscopy, tonometer, fluorophotometry and fluorescein angiography. Patients were followed-up during 6 month

**Results** In injected eyes mean preinjection fluorophotometry was 43 x10-6 ng/ml/min. After injection the value decreased of 70% after one month, and 30% after 3 and 6 months. In non-injected eyes, mean preinjection value was 36 x10-6 ng/ml/min. After injection of the other eye, the value increased of 10% at one month, 10% at 3 months and 20% at 6 months. During the same period, funduscopy showed a decrease of hyalitis, pars planitis and macular cystoid edema. Fluorescein angiography showed a decrease of the extravasation of fluorescein and of the macular edema. Average visual acuity increased of two lines in injected eyes. Ocular pressure increased of 2 mmHg at one month and 1 mmHg à 6 month. We did not observe any other side-effect

**Conclusion** The intravitreal triamcinolone acetonide injections seem to be effectives on the modifications of the blood retinal barrier permeability, therewere they are associated with low complications. Intravitreal injections of corticosteroids could become a new treatment modality in severe acute posterior uveitis. A randomized-controlled trial would be necessary to confirm this cases series

■ **4265**

## Intravitreal Triamcinolone in patients with uveitic macular edema. A prospective Study

*SCHMIDT S, RIEGER R, VELHAGEN KH, PLEYER U*
*Charité – University Berlin, Dept. of Ophthalmology, Berlin*

**Purpose** Cystoid macula edema (CME) is the most common sight threatening complication of uveitis. The mainstay of treatment has been corticosteroids, however their long term use is limited by adverse effects. Triamcinolone acetonid (TA) has gained a brought spectrum of indications, including CME. Despite its frequent application, little is know on the use of TA in chronic uveitic CME.To investigate the role of intravitreal TA we performed a prospective study analysing its effects on uveitis CME.

**Methods** 16 patients with therapy refractory CME received TA (4mg) intravitreally and were prospectively followed. Pre- and postoperative exams included ophthalmologic visual acuity (VA), intra ocular pressure (IOP), slit lamp biomicroscopy, optical coherent tomography and in selected patients angiography.

**Results** All 18 patients demonstrated improved VA and resolution of CME. During a mean follow up time of 8 months the VA remained stable in 15 patients. Between 6 weeks to 3 months cystoid spaces begun to return in some eyes but respond to reapplication of intravitreal injection. There were no severe postoperative complications like endophthalmitis, however, IOP increased in two patients late postoperatively.

**Conclusion** Intravitreal injection of TA is an effective and safe treatment option in selected patients with therapy refractory uveitic CME.

■ **4266**

## Aetiologic diagnosis in uveitis of childhood

*BOGDANICI C, ROBLI M, BRATU C, CRASMARU C*
*Dept. of Ophthalmology, Iasi*

**Purpose** To study the difficulties in establishing the causes of uveal inflamation in children.

**Methods** Retrospective study for 5 years (2000 – 2004) of 56 patients examined with uveitis and the onset of ocular diseases before the age of 16 years. Clinical parameters studied: age and sex distribution, laboratory date, the onset, course and anatomical location of ocular inflammation, therapeutic strategies.

**Results** 60,71% of cases were male and 39,28% female. Age distribution is 21,43% in the group 0 - 5 years, 26,78% in the group 6- -10 years and 51,78% in the group 11 – 16 years. Anatomical distribution of uveitis were 57,14% anterior, 3,57% intermediate, 32,14% posterior, and 7,14% panuveitis. 17,85% were bilateral. Aetiologic diagnosis founded: postoperative uveitis in 28,57% of cases, posttraumatic uveitis in 21,43% of cases, toxoplasma uveitis in 19,64% of cases, toxocara uveitis in 7,14% of cases, idiopathic uveitis in 12,5% of cases and other causes in 14,28%.

**Conclusion** Uveitis in children is a potentially blinding disease, of which the aetiological diagnosis is difficult, investigations being complex and numerous.

■ **4267 / 477**

## Contribution of lumbar puncture and CSF analysis in the diagnosis of Vogt-Koyanagi-Harada disease

*BOUCHENAKI N (1, 2), HERBORT CP (1, 2, 3)*
*(1) Memorial A. de Rothschild, Clinique Générale-Beaulieu, Geneva*
*(2) Inflammatory Eye Diseases, La Source Eye Centre, Lausanne*
*(3) Lausanne University, Lausanne*

**Purpose** Vogt-Koyanagi-Harada disease (VKH) is characterized by a bilateral granulomatous uveitis with exudative retinal detachments associated with systemic neurological, auditory, tegumentary manifestations. VKH is relatively unfrequent in Europe, therefore diagnosis is often reached with some delay. The aim was to determine the utility of lumbar puncture (CSF analysis) in the diagnosis of VKH disease in a non endemic setting.

**Methods** The charts of patients with the diagnosis of VKH seen at la Source Eye Centre in Lausanne from 1.1995 to 1.2005 were analysed. The delay from onset of the disease to diagnosis was established. Different diagnostic investigations were analysed and, in particular, their contribution toward diagnosis was evaluated. The proportion of patients were CSF analysis confirmed neurological involvement or, represented the only parameter indicating neurological involvement was analysed.

**Results** Twenty patients with VKH disease were seen in our centre. 9 patients (45%) presented neurological symptoms (headache, meningismus...) before ocular manifestations. 14 patients (70%) underwent lumbar puncture with CSF analysis and 12 (86%) presented a lymphopleocytosis. The 2 negative CSFs came from patients in the chronic stage and under treatment. In 5/12 patients (45%) CSF pleocytosis was the only neurological sign. In 3 patients, the association of CSF pleocytosis and the presence of choroidal lesions on the indocyanine green angiography (ICGA) allowed the diagnosis of VKH disease.

**Conclusion** CSF analysis can contribute to the diagnosis of VKH disease in non endemic areas, even in the absence of neurological symptoms. The association of CSF pleocytosis and the presence of choroidal signs on ICGA can help to confirm VKH diagnosis.

■ 4311

## Mapping of a recessive cataract mutation to rat chromosome 1

*GRAW J (1), PRETSCH W (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 Human Genetics, Neuherberg*

**Purpose** The cat rat was identified after parental radiation carrying an autosomal recessive mutation leading to a nuclear cataract. It can be detected in 75% of the mutants at weaning, but in 100% at the age of 70 days (Leonard and Maisin, Nature 205, 1965, 615-616). Aim of the actual study was the mapping of the mutation and the characterization of positional candidate genes.

**Methods** Homozygous cat/cat rats (on Wistar background) have been outcrossed to wild-type Brown Norway rats; heterozygous F1 rats were backcrossed to the parental mutant line. Homozygous F2 animals have been checked for the presence or absence of cataracts; linkage analysis was performed using microsatellite markers. Candidate genes have been sequenced.

**Results** In total, 461 F2 rats have been analyzed, 223 were with cataracts (48%). At weaning, 149 (67%) cataracts of the F2 rats were detected, and further 74 during the next 7 weeks (most at the age of 7-8 weeks). Among all cataractous F2 rats, 149 (67%) were white, 32 (14%) brown and 42 (19%) hooded indicating linkage of the cat mutation to rat chromosome 1 carrying the albino locus. Detailed haplotype analysis placed the cat mutation between the marker D1Rat196 and the albino locus; the genetic distances are 11.4 +/- 2.1 and 32.3 +/- 3.2 cM., respectively. Unfortunately, no other marker in the critical interval was informative. Based upon the linkage data, Lim2 and Six5 are good candidate genes to carry the causative mutation. Since sequencing data of wild-type Wistar and mutant cat/cat rats revealed no differences within the Lim2 cDNA, Lim2 was excluded as candidate gene. Analysis of the Six5 sequence is still in progress.

**Conclusion** The radiation-induced cataract mutation cat in the rat was mapped to chromosome 1; Lim2 was excluded as a candidate gene.

■ 4312

## Chromatin remodeling and crystallin gene regulation in vivo in lens

*CVEKL A (1, 2), YANG Y (2), PIRITY M (2), STOPKA T (3), CHAUHAN BK (1), SKOULTCHI A (3)*
*(1) Ophthalmology, Bronx*
*(2) Molecular Genetics, Bronx*
*(3) Cell Biology, Bronx*

**Purpose** Postmitotic differentiating lens fiber cells express very high levels of crystallins. Previous studies have shown important roles of lens lineage-specific DNA-binding transcription factors in this process, however, mechanism of crystallin gene regulation at the level of chromatin is not known. Ordered chromatin structure represents a structural barrier for transcription. To overcome this problem, gene activation in vivo requires recruitment of ATP-dependent chromosomal remodeling complexes SWI/SNF and ISWI.

**Methods** Chromatin immunoprecipitations using antibodies against catalytical subunits Brg1 (SWI/SNF) and Snf2h (SWI/SNF) of remodeling complexes in lens chromatin were performed and analyzed in a 16 kb region of the mouse aA-crystallin locus. Transgenic mice were generated to ectopically express a dominant negative form of Brg1 in lens fiber cells.

**Results** In chromatin obtained from cultured lens epithelial cells, Snf2h was found in the promoter region and small amounts of Brg1 were found in the promoter and other regions of the locus. In contrast, recruitment of high amounts of both Brg1 and Snf2h were found across the analyzed 16 kb region of the mouse aA-crystallin locus in chromatin obtained from P1 mouse lens. Expression of enzymatically inactive Brg1 in lens fibers of three transgenic lines resulted in abnormal lens fiber cell differentiation.

**Conclusion** Limited recruitment of Snf2h to the aA-crystallin promoter in cultured lens epithelial cells and broad recruitment of both Brg1 and Snf2h to the mouse aA-crystallin locus suggest an important role of chromatin remodeling for high aA-crystallin expression in lens fiber cells.

■ 4313

## Loss of Chaperone Function of Alpha-Crystallin: A Probable Cause of Diabetic Cataract

*ABRAHAM EC*
*Biochemistry and Molecular Biology, Univ of Arkansas for Medical Sciences, Little Rock, AR*

**Purpose** The purpose of the study was to show the role of glycation and truncation of C-terminal residues of alpha-crystallin in the loss of chaperone activity seen in diabetic lenses.

**Methods** Alpha-crystallin was prepared from diabetic and nondiabetic rat lenses and from 52-56 and 63-69 year-old diabetic and non-diabetic human lenses and analysed by electrospray ionization mass spectrometry (ESIMS). Recombinant truncated alphaA-crystallins were expressed in E. coli and purified by molecular sive HPLC. The lysine (K)residues, previously shown to be glycation sites, were mutated to threonine (T). Glycation and AGEs were measured by the affinity method and animmunochemical method, respectively. Chaperone assays were done with ADH as the target protein.

**Results** Diabetic rats showed nearly 50% loss in chaperone activity. A strong correlation between chaperone activity loss and plasma glucose levels was evident. Alpha-crystallin from diabetic human lenses also showed significant loss in chaperone activity. ESIMS analysis showed the presence of several C-terminal truncated alphaA-crystallins, whose levels were substantially increased in diabetic lenses. Chaperone activity of several of the truncated alphaA-crystallins was decreased by 50-100% because of altered oligomeric structure. K to T mutations showed significant inhibition of glycation and a corresponding protection from chaperone activity loss due to glycation.

**Conclusion** High level of structurally and functionally altered truncated alpha-crytallins and enhanced glycation of alpha-crystallin significantly affect chaperone activity. This in turn is expected to increase protein aggregation and cataractogenesis. (Supported by NIH Grants EY07394 and EY11352)

■ 4314

## Inhibition of GSK-3 protects the lens against oxidative stress

*KARLSSON JO, PETERSEN A, SOLTANPOUR Y*
*Institute of Anatomy and Cell Biology, Univ of Göteborg, Göteborg*

**Purpose** GSK-3 is an evolutionary conserved S/T kinase which regulates cell fate determination in diverse organisms.GSK-3 has been implicated in the formation of amyloid b-peptides and the phosphorylation of tau and catenin. Inhibition of GSK-3 can be obtained via the structurally unrelated substances lithum or Kenpaullone.

**Methods** Confluent human lens epithelial cells (HLEC) or the whole mouse lens in organ culture were exposed to the GSK-3 inhibitors lithium (2 mM) or Kenpaullone (2 µM) for times upp to 24h.Proteasome and calpain activities were assayed with LLVY-AMC. Cathepsin B: RR-AMC or FR-AMC. Cathepsin D/E: Ac-RGFFP-AMC. Metalloproteases were assayed with AAF-AMC. Caspase-3, 8 and 9 were assayed in cell extracts with DEVD-, IETD- or LEHD-AMC, respectively.Peroxides: DCFH-DA Mitochondrial potential: JC-1 and Rhodamine 123.Superoxide: Hydroethidine (HET)GSH (Glutathione): Monochlorobimane

**Results** The mitochondrial membrane potential as measured by JC-1 or Rhodamine 123 increased after relatively low doses of of Lithium or kenpaullone. This was observed in primary culture of human lens epithelial cells as well as in the mouse lens in organ culture. The level of GSH also increased.The basal (low) level of caspase-3 acyivity was decreased in HLEC. No change was observed for the activities of other major proteolytic systems in the lens were found after treatment.

**Conclusion** Inhibition of GSK-3 may protect against oxidative damage and attenuate apoptosis in HLEC. No changes of the other major proteolytic systems in the cell were detected. These data may be important for the interpretation of Wnt signaling and cell growth in HLEC as well as for the formation of amyloid in the lens.

■ **4315**

## Regulation of intercellular communication in lens epithelial cells: the role of C-Terminus of connexin 43

*GIRAO H, RAMALHO J, CATARINO S, PEREIRA P*
*IBILI - Centre of Ophthalmology, Faculty of Medicine, University of Coimbra, Coimbra*

**Purpose** Intercellular communication through GAP junctions (GJIC) in lens epithelial cells is vital to maintain lens transparency. Phosphorylation of the carboxyl-terminus (CT) of connexin43 (Cx43) has been suggested to trigger its degradation. The objective of this study is to investigate the role of the CT in the regulation of Cx43 stability at the plasma membrane of lens epithelial cells

**Methods** Lens epithelial cells were incubated with protein kinase activator (TPA) and allowed to recover either in the presence or absence of proteasome inhibitors. The contribution of the proteasome for the degradation of the phosphorylated form of Cx43 was evaluated by pulse chase experiments with 32P. The role of the CT for proteasomal dependent degradation of Cx43 was assessed using truncated forms of Cx43 fused to GFP. Subcellular distribution of Cx43 was evaluated by immunofluorescence confocal microscopy. GJIC was evaluated by transfer of the dye Lucifer yellow.

**Results** Pulse chase experiments showed that phophorylation increases the turnover of Cx43, whereas proteasome inhibitors selectively stabilise phosphorylated form of Cx43. Moreover, proteasome inhibitors restore intercellular comunication following inhibition by TPA by preventing internalization of Cx43 by stabilizing full length Cx43 at the plasma membrane. Significantly, truncated forms of Cx43 at the CT are not affected by proteasome inhibitors

**Conclusion** This study supports a model in which phosphorylation may act as a triggering signal for the internalization of Cx43 by a proteasome-dependent mechanism. Phosphorylation and proteasome-dependent degradation of CX43 may be novel mechanism regulating intercellular communication in the lens.

## ▪ 4321
## Biofluidmechanical simulation for age-dependent local retinal arterial reaction

*KOTLIAR KE (1), SCHILLING R (2), ANCIGER D (2), EINZINGER J (2), VILSER W (3), LANZL IM (1)*
*(1) Ophthalmology, Munich University of Technology, Munich*
*(2) Department of Fluid Mechanics, Munich University of Technology, Munich*
*(3) Biomedical Engineering, Ilmenau University of Technology, Ilmenau*

**Purpose** Using image analysis by Retinal Vessel Analyser it is possible to assess vessels in their dynamic state online non-invasively along a chosen vessel segment. Previously we investigated the vessel longitudinal section of retinal arterial segments of 1 mm length in healthy persons of different age. We found that the high-frequency waviness of retinal arterial walls (HFW) along a vessel longitudinal section increases significantly in anamnesticaly healthy volunteers with increasing age. It seems that vessels in the elderly assume a less regular profile, which might be an expression of endothelial damage. Whether these changes might influence hydraulic parameters of a vessel was investigated in the presented study using methods of computational fluid dynamics (CFD).

**Methods** A C++ program was written in order to create 3-D geometry of a retinal arterial segment and corresponding computational grid. This allowed us to vary parameters of HFW. Finite Volume Modelling procedure was applied for further calculations with CFD-code NS3DV43C. Pressure drop along an arterial segment was calculated in dependence to HFW with the assumption that the volumetric flow through the vessel cross-section remains constant.

**Results** Pressure drop along the arterial segment increased with increasing HFW. Consequently, age-related increase in AHW worsened hydraulic conductivity of retinal arteries.

**Conclusion** Retinal branch vessels possess alternating vessel diameters which constitute the vessel longitudinal section profile. Our results show that increasing HFW worsens hydraulic conductivity in anamnestically healthy volunteers with increasing age. This might be the reason for age related ocular as well as systemic vascular disorders.

## ▪ 4322
## A reassessment of Fisher's spinning lens test

*BURD HJ (1), WILDE GS (2), JUDGE SJ (2)*
*(1) Department of Engineering Science, Oxford University, Oxford*
*(2) University Laboratory of Physiology, Oxford University, Oxford*

**Purpose** Accurate data on lens stiffness are needed for detailed modelling of accommodation and presbyopia. However, current published data indicate substantial differences in reported values of lens Young's modulus. A study has therefore been undertaken on one source of data (Fisher 1971) to investigate the reliability of the quoted values of Young's modulus.

**Methods** Several of the assumptions employed in the analytical model of Fisher (1971) have been investigated and new analytical and finite element models have been developed. Preliminary spinning lens tests have also been conducted on the porcine lens to investigate the structural action of the capsule.

**Results** (a) Spinning disc models (as employed by Fisher) induce systematic errors into the determination of the cortex and nucleus Young's modulus. These errors tend to lead to values of inferred modulus ratio (Young's modulus of nucleus / Young's modulus of cortex) that are artificially low.(b) An alternative modelling approach suggests that Fisher's data could be interpreted in terms of an elastic lens with a single value of Young's modulus.(c) 3D finite element studies indicate that the effect of lens alignment errors are likely to be minimal.(d) In contrast to Fisher's observation on a human lens, preliminary tests show that the capsule has a substantial effect on the deformations generated in the spinning lens test for a porcine lens.

**Conclusion** One puzzling feature of Fisher's results (that the cortex generally appears to be stiffer than the nucleus) is shown to be attributable, at least in part, to systematic errors in the original interpretation process. Fisher's observation that the capsule has a minimal effect on the deformations generated in the spinning lens test, however, requires further investigation.

## ▪ 4323
## Effect of Intravitreal Injections and Volume Changes on Intraocular Pressure – a Biomechanical Model

*MAIER M (1), LANZL IM (1), BAUER SM (2), FEUCHT N (1), FISCHER E (1), WINKLER VON MOHRENFELS C (1), LOHMANN C (1), KOTLIAR KE (1)*
*(1) Ophthalmology, Munich University of Technology, Munich*
*(2) Mechanical Engineering, State University, St.Petersburg*

**Purpose** Intravitreal injections (IVI) have been extensively used to treat retinal diseases. When applying an IVI the intraocular volume is increased by the amount of fluid brought into the eye. To calculate the effect of different volume changes on intraocular pressure (IOP) a biomechanical model is used.

**Methods** The model was applied to calculate the short term effect on IOP after IVI. We calculated the effect after intravitreal injection of 0,1 ml and 0,2 ml for hypermetropic, emmetropic and hyperopic eyes. Our calculations were compared with IOP measurements obtained immediately after IVI of 4mg/0,1ml Triamcinolone (IVTA). Briefly after the measurement the IOP was reduced through a paracentesis to 15 mmHg.

**Results** The calculation of the expectable IOP elevation and the measurements were comparable. Immediately after IVI of 4mg/0,1ml Triamcinolone IOP was elevated up to 80mmHg. The measured and the calculated IOP was comparable. In hypermetropic eyes the calculated and measured IOP-increase was highest.

**Conclusion** With a biomechanical model the effect of different injected volumes on IOP can be calculated. Since IOP increases are counterregulated by increased outflow volumes in non glaucomatous eyes this increase in IOP especially in myopic eyes might be only present for a short term period. IVI are mostly applied in diseases which are due to vascular compromise. So even a short term increase in IOP might be able to impair the already dysfunctional perfusion. The results of our calculations and the short term measurements of IOP show that a pracentesis is recommended after injection to avoid high IOP levels especially in short hyperopic eyes.

## ▪ 4324
## Biomechanical interrelation between the lamina cribrosa topography and the tone of the ciliary muscle in myopia

*RYABTSEVA AA (1), HOMJAKOVA EN (1), SERGUSHEV SG (1), SVETLOVA OV (2)*
*(1) Department of Ophthalmology, Moscow Region Scientific-Reaserch Medical Institute, Moscow*
*(2) Department of Ophthalmology, Medical Academy of Postgraduate Education, St.Petersburg*

**Purpose** In the human eye there are several extraocular and intraocular mechanisms of the accommodation. During accommodation the ciliary muscle (CM) contracts and the tension of zonular fibers decreases. Consequently, the pressure in the closed hyaloid chamber (PHC) decreases. It increases during desaccommadattion (Svetlova et al., 2004) and in this case the additional excavation of the lamina cribrosa (LC) may happen. The mechanical strength of the LC in myopia seems to be reduced due to the stretching of the scleral posterior pole. Thus the increase of excavation becomes more visible.

**Methods** Three groups of patients were investigated: I: myopia of high level (27 eyes), II: myopia of medium and low level + POAG with normal IOP (18 eyes), III: POAG (35 eyes). The topography of the LC was analyzed using HRT II at usual (medium) tone of accommodation and after instillation of the Cyclomed 1% at desaccommodation.

**Results** Cyclomed increased the average/maximum depth of LC excavation in the I group to 0,010/0,041mm in the II: to 0,015/0,022mm, in the III: to 0,044/0,092mm correspondingly. LC excavation volume increased in the I group to 0,013mm (II: to 0,054mm, III: to 0,048mm). The ratio of excavation area to the LC - area increased in the I group to 0,026mm (II: to 0,037mm, III: to 0,076mm).

**Conclusion** The sensitivity of the method allows to estimate the residual working capacity of the LC supporting mechanism at anatomically normal position during the changes in IOP and in CM tone as well as the efficiency level of the hypotensive medical therapy.

■ 4325

## Study of Biomechanical Properties of the Sclera Using Different Methods

*IOMDINA EN*
*Laboratory of Myopic Studies, Helmholtz Research Institute of Eye Diseases, Moscow*

**Purpose** Biomechanical parameters of the corneoscleral shell of the eye have not been thoroughly studied so literary data are often contradictory. This may be due both to biomechanical particularities of the shell itself and the application of diverse techniques of study. The goal is to evaluate the potential of diverse methods of studying the elasticity modulus (E) of isolated sclera.

**Methods** Uniform-size samples of the sclera of 28 rabbit eyes, 6 pig eyes and 24 healthy eyes of humans aged 20-55 cut from different areas of the shell in longitudinal and transverse directions were studied on the Instron 1029 testing machine (allowing one to produce the diagram of relation between the sample elongation and the load applied) and the Minimat 2000 machine supplied with a microcomputer and producing the stress-strain dependency. In both cases, the loading rate was 1 mm/min.

**Results** The data obtained are essentially different depending on the location of the sample and its orientation with regard to the anterioposterior axis of the eye. If these factors are considered, the elasticity modulus of rabbit sclera varies between 5.0-23.6 MPa (Instron data) and 3.5 - 48 MPa (Minimat Data); pig sclera is characterized by a higher value of E: 26.0-57.0 MPa (Minimat). The elasticity modulus of isolated human sclera varies from 12 to 47 MPa (Instron data) and from 2.5 to 26 MPa (Minimat data).

**Conclusion** Deviation of biomechanical testing data is accounted for by specific conditions of sample loading as well as an essential anisotropy and heterogeneity of the scleral shell, which is especially notable in human sclera. The use of the Minimat 2000 machine may be considered the most adequate for biomechanical studies of scleral tissue.

■ 4326

## Physiological and biomechanical principles of the hypotensive medical therapy in presbyopia

*KOSHITZ IN (1), KOTLIAR KE (2), ZASEEVA MV (3), SVETLOVA OV (3)*
*(1) Petercom MS/Consulting group. Inc, St.Petersburg*
*(2) Department of Ophthalmology, Munich University of Technology, Munich*
*(3) Medical Academy of Postgraduate Education, St.Petersburg*

**Purpose** When primary open angle glaucoma (POAG) has been diagnosed the medical hypotensive therapy is usually the first method of treatment. Surgery or laser surgery represent the next step if medications don't work properly. However often the contemporary hypotensive therapy provides the temporarily inhibition of glaucomatous process and sometimes led to inexplicable paradoxical results.

**Methods** Basing on control theory and biomechanical analysis of the clinical and experimental data of last 60 years physiological principles of interdependent functioning of the accommodation, aqueous production and aqueous drainage are formulated. These are applied to elaborate pathogenetically adequate approaches for effective hypotensive mono- and combining therapy of POAG.

**Results** Generalised practical recommendations for the application of pharmacological hypotensive therapy in presbyopic period are elaborated, which allows not only to reduce the influence of unwanted effects but also shows perspective ways of the investigations for elaborations of the new hypotensive drugs.

**Conclusion** Type, intensity, duration, combination and sequence of the hypotensive pharmacotherapy need to be chosen depend on the type of actual optical refraction of the eye and especially on its declination from emmetropia. The elaborated individual physiological principles of the consecutive and step-wise use of the hypotensive pharmacotherapy might allow to improve its effectiveness and to exclude negative and paradoxical cases of its application.

■ 4331

## Results of a diabetic retinopathy screening programme in London

*PETO T, HARRIS N, LEUNG I, ANDREWS R*
*Moorfields Eye Hospital, London*

**Purpose** Diabetic retinopathy (DR) is the leading cause of blindness in the working age-group in the developed world. It has been established that screening for DR can save sight. In England, a government-led initiative, the National Service Framework (NSF), provides guidelines for screening for DR using digital photography. The Reading Centre at Moorfields Eye Hospital, London, England plays a vital role in setting up these programs by training personnel, provide the service and carry out quality control.

**Methods** This study analyses the first year's screening results in an East London DR Screening Service, based on the NSF's 15 criteria, these cover blindness statistics, database issues, quality of the photographs, grading and clinical services.

**Results** During the first year of the service 2802 patients with diabetes have been photographed by a nurse and a technician. The photos were of good enough quality in 99% to place the patient into the appropriate clinical pathway. The most common cause of poor quality was cataract, these patients were referred for surgery. Overall 117 patients (4.2%) required referral for DR, most of them had maculopathy (87). Proliferative DR was diagnosed in 3 patients, all of whom received laser treatment within 2 weeks of diagnosis.

**Conclusion** In summary, this Reading Centre led screening program for DR provided good quality service to the patients based on the strict quality control criteria. It screened out those needing ophthalmic services while reassured the 95.8% of patients who did not require ophthalmic follow-up. Reducing the number of patients in the specilised ophthalmic clinics meant that patients referred were treated promptly and efficiently.

■ 4332

## OCT findings in DM type 1 patients with minimal or no retinopahy

*VERBRAAK FD (1, 2), VAN VELTHOVEN MEJ (1), SCHLINGEMANN RO (1), MICHELS BPJ (3), DE VRIES HH (3)*
*(1) Academic Medical Center, Dpt of Ophthalmology, Amsterdam*
*(2) Academic Medical Center, Laser Center, Amsterdam*
*(3) Academic Medical Center, Dpt of Internal Medicine, Amsterdam*

**Purpose** To evaluate the retinal thickness and the changes in retinal thickness over time, measured with the OCT-Stratus in patients with type 1 DM, and no or minimal DRP, related to parameters of metabolic control.

**Methods** Fifty consecutive patients with type 1 DM, seen at the internal department of the Academic Medical Center, without known DRP changes, were also examined with the OCT, once every 4 months. At the same time stereo photographes were made of the macula, and patients underwent a full general ophthalmological examination. Data were collected simultaneously of the metabolic control (HbA1c, cholersterol, triglycerides, TSH, kreatinine clearance, bloodpressure, duration of disease, medications used).

**Results** Most patients did not show any visible sign of DRP, neither on biomicroscopy or stereophotographs. Retinal thickness was whithin normal limits for all patients. All fifty patients were at least seen twice, 30 patients three times, and 15 patients four times. Retinal thickness remained within normal limits throughout the available follow-up time.Differentiating between patients with good (HbA1c < 7.0) and moderate to poor (HbA1c > 7.0) control of the DM, we did find a tendency towards thicker retinas in the patients with poor control.

**Conclusion** The retinal thickness maesured with OCT in patients with type 1 DM, and no or minimal DRP, is whithin normal limits. During a relative short follow-up the retinal thickness remained whithin normal limits, although there seems to be a tendeny towards thicker retinas related to poor metabolic control.

■ 4333

## Intravitreal triamcinolone acetonide in the treatment of severe bilateral diabetic cystoid macular edema

*SUMMANEN PA, KYTÖ JP, LUMISTE E*
*Dept. of Ophthalmology, University of Helsinki, Helsinki*

**Purpose** To study the long-term effect of intravitreal triamcinolone acetonide in severe bilateral diabetic cystoid macular edema (CME).

**Methods** Diabetic patients with low vision (visual acuity, VA <0,3 in the better eye) due to persistent bilateral CME after standard focal and extrafoveal grid laser treatment were eligible. Before treatment fluorescein angiogram and optical coherence tomography were performed and VA tested with Snellen and ETDRS-chart. Antibiotic drops were instilled, the patient prepated and draped in an outpatient surgical unit and 0,1 ml triamcinolone acetonide solution was injected into vitreous cavity. Follow-up examinations took place at 4 days, 2 weeks, and every month for one year and every third month thereafter.

**Results** Between 10/2003 and 11/2004, nine patients (M/F=6/3) with type 1/2 diabetes (4/5) were eligible to the study. All used insulin and 7 were on antihypertensive medication. Primarily the eye with lower vision was chosen to treatment, but due to positive response the other eye was treated in three patients (altogether 12 eyes). The mean number of letters (+-standard deviation) read at 2 meter increased from the preoperative 15,2+-11,4 to 30,8+-11,7 at 2 months. In the fellow-eye the average number of letters remained unchanged (25-27 letters). During the median follow-up of 1 year and 4 months (range 6 months to 2,5 yrs) the central retinal thickness decreased markedly in 4, slightly in 5 and remained unchanged in 3 eyes. Intraocular pressure increased in 8 out of 12 eyes and posterior subcapsular cataract developed in 6 out of 10 phakic eyes.

**Conclusion** Intravitreal triamcinolone may be used as an adjuvant therapy for persistent severe cystoid macular edema.

■ 4334

## Intravitreal triamcinolone for the treatment of idiopathic juxtafoveal retinal telangiectasis

*YILMAZ G, GEDIK S, AYNACI B, AKKOYUN I, AKOVA Y*
*Department of Ophthalmology, Baskent University School of Medicine, Ankara*

**Purpose** To evaluate the clinical outcome of the intravitreal injection of triamcinolone acetonide (TA) for treatment of macular edema due to idiopathic juxtafoveal retinal telangiectasis (IJRT).

**Methods** We studied retrospectively 22 eyes of 11 patients with diagnosis of clinically significant macular edema due to IJRT. The patients were given intravitreal injection of TA (4mg/0.1 ml). Visual acuity, intraocular pressure (IOP) measurements and fundus fluorescein angiography (FFA) findings were recorded before the drug injection and throughout follow-up period.

**Results** Two patients were male and 9 were female. The patients had a mean follow-up period of 7.2+/-2.3 months. Eight patients showed a significant clinical resolution of macular edema and decrease of fluorescein leakage on FFA at an average time of 3 weeks. Visual acuity increased from 0.4+/-0.1 to a maximum of 0.7+/-0.2 during the follow-up period. However, 3 patients did not show any improvement in terms of visual acuity and fluorescein leakage. Two patients with an IOP over 30mm Hg received topical antiglaucomatous treatment and IOP control was achieved during the follow-up period. No other complications were noted.

**Conclusion** Intravitreal injections of TA may be benifical to improve visual acuity in patients with macular edema due to idiopathic juxtafoveal retinal telangiectasis.

■ 4335 / 449

## Audit of diabetic referrals – Rationalising patient referrals via a three-tier system

*FLETCHER EC, ADEWOYIN TA, CHONG NHV*
*Kings College Hospital, London*

**Purpose** Screening services for diabetic retinopathy provide an invaluable facility aiming to ensure appropriate referral of patients to the retina specialist centres. We would like to describe a modified and enhanced service running in King's College Hospital, which enables suitable and timely referrals.

**Methods** Annual retinal screening is performed using retinal photography by the Diabetic Eye Complication Screening Service. A trained level 1 screener / photographer interprets the photographs on the spot to assess whether the images were normal and acceptable. Abnormal images are then passed on to the level 2 screener for referral decision. The treating retinal specialist can assess the referred images using the same software. If referral is not deemed necessary at this stage then advice regarding further screening is given and the patient informed of the outcome.

**Results** Of the 2260 patients screened between Nov 2004 to April 2005, referral was requested on 186 (8.2 %). Of those, 94 (50.5 %) were accepted for examination in the retina unit for possible treatment. The most common reasons that further assessment were not required were early maculopathy without clinically significant macular oedema, fundal lesions of no consequence and previously treated but no active maculopathy.

**Conclusion** Diabetic retinopathy screening has already been proven to provide both a sensitive and specific method for detection of sight threatening diabetic retinopathy. This three tier method reduces referrals by ~50% and thus forms a streamlined service ensuring that busy retinal clinics are used effectively and provides an improved service for patients who can remain under close observation in their local area.

■ 4336 / 450

## Detection of associated ocular lesions during screening of diabetic retinopathy

*DEB-JOARDAR N (1), THURET G (1), GERMAIN N (2), GARCIN AF (1), MANOLI P (1), GAVET Y (3), ESTOUR B (2), GAIN P (1)*
*(1) Department of Ophthalmology, University Hospital, Saint-Etienne*
*(2) Department of Endocrinology, university hospital, Saint-Etienne*
*(3) Ecole nationale supérieure des Mines, Saint-Etienne*

**Purpose** Screening for diabetic retinopathy (DR) is insufficient in France mainly due to an increased incidence of diabetes and lack of enough ophthalmologists. In this setting, digital camera provides a sensitive and cost-effective option. Apart from DR, this allowed to detect other coexisting pathologies

**Methods** We prospectively recorded the fundus photos of 1153 consecutive patients during 1 year. Patients underwent five-field (45°) imaging through pharmacologically dilated pupils (Topcon TRC NW6S) followed by specialist interpretation. Patients were interrogated for antecedent history and referred for further evaluation

**Results** The patients (578 males, 575 females) aged 57±16 years had a 14±11 years evolution of diabetes. Coexisting pathologies were recognized in 612 patients (53%). The most common associated lesions were hypertensive retinopathy (n=205, 18%), significant cataract (n=176, 15%), age-related macular degeneration (ARMD) (n=66, 6%) and optic nerve excavation (n=33, 3%). Twenty-three patients (2%) had previously undiagnosed sight-threatening lesions: ARMD (n=4), retinal vein occlusions (n=4), florid hypertensive retinopathy (n=3), macroaneurysms (n=2), macular hole (n=1), rhegmatogeneous retinal detachment (n=1), retinitis pigmentosa (n=1) and choroidal mass lesion (n=1). Significant optic nerve lesions detected were papilloedema due to intracranial metastasis (n=1), optic atrophy (n=2, pituitary tumour, nonarteritic neuropathy) and glaucomatous cupping (n=1)

**Conclusion** Multiple potentially sight-threatening lesions apart from DR were detected during routine screening. Quality interpretation and timely referrals may help to prevent possible visual loss

■ 4337 / 451

## A two-year prospective study of mild nonproliferative retinopathy in subjects with diabetes type 2 undergoing intensive oral tritherapy. Progression of retinopathy under stabilized metabolic control

*DUARTE L (1), RIBEIRO ML (1), NUNES S (1), LOBO C (1, 2), BERNARDES R (1), GERALDES E (3), FARIA DE ABREU JR (2), CUNHA-VAZ JG (1, 2)*
*(1) AIBILI – Association for Innovation and Biomedical Research on Light and Image, Coimbra*
*(2) Department of Ophthalmology, University Hospital, Coimbra*
*(3) Department of Endocrinology, University Hospital, Coimbra*

**Purpose** To evaluate progression of mild nonproliferative retinopathy in subjects with diabetes type 2 under stabilized metabolic control

**Methods** Forty-five type 2 diabetic patients with mild nonproliferative retinopathy were treated with an association of three hypoglycemic agents (biguanide, alpha-glucosidase inhibitor, sulphonylurea) to optimize their metabolic control. Patients were followed-up prospectively for a two-year period.

**Results** Metabolic control remained stabilized with an overall decrease in HbA1C levels from 7.5% ± 1.2% to 7.4% ± 1.6%. The number of red dots in the fundus of the eye (microaneurysms + hemorrhages), however, showed significant overall increases at every visit. The rates of progression of number of red dots per year varied among patients, showing a correlation with the rate of progression before enrollment ($p = 0.007$) and increasing with HbA1C levels > 7.1% ($p < 0.001$).The number of red dots correlated with changes in the permeability of the blood-retinal barrier (BRB) and alterations in the foveal avascular zone (FAZ) when present

**Conclusion** Progression of retinopathy in type 2 diabetes during a two-year follow-up period, under stabilized metabolic control, varies among patients. Progression is more marked in eyes with more pathology at baseline and in patients presenting higher HbA1C levels.

■ 4338 / 452

## Subtenon triamcinolone acetonide to treat diabetic macular edema

*CELLINI M, ZAMPARINI E, BRAVETTI GO, BELLUSCI C, CAMPOS EC*
*Department of Surgery and Transplant "A. Valsalva," Bologna*

**Purpose** To evaluate the efficacy of Triamcinolone Acetonide subtenon injections in the treatment of diabetic macular edema.

**Methods** We selected 11 patients, 8 of which were male, and 3 of which were female, ages ranging between 61 and 74 years old (average 68.3). The patients were all affected by diabetes type II and they presented macular edema previously diagnosed with fluorescence angiography. The patients' visual acuity was evaluated utilizing the ETDRS logarithm (logMAR), the measurement of intraocular pressure (IOP), and a biomicroscopic examination of both anterior and posterior segments of the eyes. Subsequently, an Optical Coherence Tomography (OCT) was performed using an OCT3 (Zeiss-Humphrey, Dublin, CA).After a topical 0.4% oxybuprocaine anesthesia, the patients were given a 40 mg peribulbar inferotemporal subtenon injection of Triamcinolone Acetonide. Each patient was given three injections; each injection separated by 30 days. After each injection, the patients were prescribed a topical antibiotic treatment for three consecutive days.

**Results** Visual acuity before cortisonic treatment was 0.464+0.112 log with a 16.182+1.4011 mm/Hg. A month after the beginning of the treatment, visual acuity was 0.118+0.125 log ($p<0.001$) while the IOP was 16.727+1.191 mm/Hg ($p<0.147$). Twelve months after the end of the treatment, we observed a stabilization of visual acuity and IOP. After three months of treatment, the condition was successfully cured. The OCT results were also stable a year after the treatment.

**Conclusion** Utilizing Triamcinolone Acetonide with a peribulbar subtenon injection showed to be an effective solution for diabetic macular edema without showing signs of the complications.

## 4341
## Clinical and investigational approach to dark blindness

*HAWLINA M*
*University Eye Clinic, Ljubljana, Slovenia*

**Purpose** To advise how to assess patients with problems to see in the dark environment.

**Methods** Acuity, visual field measurements, adaptometry, color vision and use of electrodiagnostics will be discussed in various clinical cases. Autofluorescence imaging will be added as an important non-invasive clinical tool that can give structural and functional information, mostly in relation to PERG and mfERG and ON-OFF responses.

**Results** Typical changes in ERG will be outlined recording and recording of the macular responses at an early stage and later. Comparison will be outlined between structural and functional aspects of the examination.

**Conclusion** Night blindness may be an insidious condition that may not manifest itself initially. Besides retinal causes, it may be presenting or accompanying sign of MAR, CAR, chloroquine intake, or malabsorbtion syndromes.

## 4342
## Congenital Night Blindness

*LEROY BP (1, 2)*
*(1) Department of Ophthalmology, Ghent University Hospital, Ghent*
*(2) Center for Medical Genetics, Ghent University Hospital, Ghent*

**Purpose** To describe the phenotypes and genotypes of genetically determined night blindness.

**Methods** A case presentation format will be used to illustrate different genetically determined conditions leading to night blindness. Both clinical and electrophysiological phenotypes as well as genotypes will be discussed.

**Results** Phenotypes and genotypes of genetically determined diseases leading to night blindness are very different. An important distinction to be made is the one between stationary and progressive diseases. Indeed, other than night blindness the visual outcome differs considerably between different conditions.

**Conclusion** Genetically determined night blindness is diverse. Visual electrophysiology allows an important distinction between progressive and stationary conditions.

## 4343
## What limits normal visual performance in the dark?

*BARBUR JL*
*The Henry Wellcome Laboratories for Vision Sciences, London*

**Purpose** We examined how visual performance at lower light levels is affected by changes in the spatial and temporal properties of the retina and / or changes in the quality of the retinal image as a result of increased aberrations and scattered light. The aim of the study was to establish the extent to which retinal and / or optical factors set the limits of visual performance at low light levels.

**Methods** Pupil size, ocular aberrations, scattered light, chromatic sensitivity and "functional" contrast acuity (Aviat.Space.Environ.Med., 74,551-559, 2003) were measured as a function of retinal illuminance in the range 2 to −1 log trolands. The rms wavefront aberration was computed for the subject's mean pupil size at each light level. A new, closed-loop system was developed capable of maintaining a constant retinal illuminance, independent of pupil size.

**Results** Both the amount of scattered light and the rms wavefront aberrations were found to increase rapidly with decreasing light level in the mesopic range. This was paralleled by a massive increase in contrast acuity thresholds and a rapid loss of both red-green and blue-yellow chromatic sensitivity. There was, however, little correlation between rms wavefront aberration and contrast acuity thresholds in the low mesopic range.

**Conclusion** Increased higher order aberrations can cause a reduction in contrast acuity when the pupil size is large and vision is still dominated by cone photoreceptors signals. When vision is dominated by rod signals, contrast acuity thresholds are much increased, but the limiting factor becomes the resolving power of the retina. Visual performance in this range is strongly affected by even small changes in retinal illuminance caused by inter subject differences or within subject fluctuations in pupil size.

## 4344
## Electrophysiological diagnosis in night blindness

*HOLDER GE*
*Moorfields Eye Hospital, London*

**Purpose** To describe the electrophysiological investigation of acquired and inherited causes of night blindness.

**Methods** ISCEV Standard electroretinography (ERG: The International Society for Clinical Electrophysiology of Vision), supplemented by pattern electroretinography (PERG); ON- and OFF- rseponse recording; S-cone ERG recording; and other supplementary ERG techniques.

**Results** The diagnostic features of various inherited and acquired diseases will be described, including photoreceptor dystrophies, congenital stationary night blindness, fundus albipunctatus, melanoma associated retinopathy, vitamin A deficiency and others.

**Conclusion** Electrophysiological assessment is fundamental to the diagnosis and management of patients with night blindness.

■ 4351

## Histopathological vs clinical prognostic factors in conjunctival melanoma

*ALJAMAL RT, TUOMAALA S, KIVELÄ T*
*Department of Ophthalmology, Helsinki*

**Purpose** To study the role of histopathologic prognostic factors relative to clinical ones in predicting recurrence and survival after treatment of primary conjunctival melanoma.

**Methods** A nation-wide search identified 85 patients diagnosed with primary conjunctival melanoma in Finland from 1967 to 2000, all of whom were enrolled. Follow-up data until November 2004 were collected from the Finnish Cancer and Population Registry and patient charts. Histopathologic characteristics of the tumour were recorded and time to first local recurrence and death were analyzed.

**Results** Cell type (26% spindle, 47% mixed or indeterminate, 26% epithelioid; P=.18), presence of extravascular networks (42%; P=.36), microvascular density (MVD; P=.70), infiltrating lymphocytes (42% few, 58% moderate to many; P=.34) and macrophages (54% few to moderate, 46% many; P=.40) were not associated with local recurrence. Of clinical variables, only tumor site was associated with first recurrence (P=.006). The cell type (P=.20), networks (P=.76), MVD (P=.89), lymphocytes (P=.17) and macrophages (P=.97) were not associated with melanoma-related survival. Of clinical variables, tumor site (P=.0002), thickness (P=.009) and largest diameter (P=.003) were strongly associated with tumour death.

**Conclusion** Unlike is the case with uveal melanoma, cell type, MVD, extravascular matrix networks and tumour- infiltrating macrophages were not associated with survival after conjunctival melanoma. None of these variables predicted local recurrence of the tumour either. The findings suggest that the biological behavior and dissemination of conjunctival melanoma differ from those of uveal melanoma. Clinical predictors, tumour site and size, remain strong prognostic factors for conjunctival melanoma.

■ 4352

## Incidence of intra ocular lymphoma in Leuven

*VAN GINDERDEUREN R, VERHAMME T, VAN CALSTER J*
*ophthalmology, Leuven*

**Purpose** Intra ocular lymphoma is a rare but malignant tumour with a severe prognosis. The insidious beginning phase is difficult to distinguish from other causes of vitritis. Once the diagnosis is established treatment options are spare and life expectancy is known to be very short.

**Methods** We retrospectively reviewed all the cases in which a diagnostic vitrectomy was performed for possibility of intra ocular lymphoma. 40 diagnostic vitrectomies were performed between 1996 and 2004 in our department and analysed for this reason.

**Results** In 11 patients the presumptive diagnosis of intra ocular lymphoma was made after biopsy screening; in ten patients the material was not sufficient for definitive conclusion of lymphoma because of too few or too necrotic cells to perform immunologic staining. In three patients the diagnosis was delayed for this reason. Intra ocular lymphoma was or became part of central nervous system lymphoma in 7 cases.

**Conclusion** Intra ocular lymphoma is a rare but fatal tumour; diagnosis is a problem even with modern techniques;

■ 4353

## Assessment of clonal heterogeneity in choroidal melanoma by chromosome in situ hybridization for chromosome 3

*SANDINHA MT (1), FARQUHARSON M (2), ROBERTS F (2)*
*(1) University Department of Ophthalmology, Glasgow*
*(2) University Department of Pathology, Glasgow*

**Purpose** Recent cytogenetic research has shown that uveal melanomas cluster into two discrete molecular classes (1 and 2) and that these correlate with cell type. Class 1 corresponds to spindle cell melanomas and class 2 to melanomas with more epithelioid cells. Many genes on chromosome 3 are down-regulated. We aimed to identify a morphological correlation with monosomy 3 by determining copy number of chromosome 3 within spindle cell and epithelioid cell rich areas of choroidal melanoma.

**Methods** We identified 22 cases of choroidal melanoma in which discrete populations of spindle and epithelioid cells were dentified. Nineteen cases were from patients who had died from metastatic melanoma and 3 were from patients alive or dead from other causes. Chromosome in situ hybridization was used to study monosomy 3 in the whole tumor and in spindle and epithelioid areas.

**Results** Twelve of the 19 cases of metastasizing melanoma were monosomic for chromosome 3. When the different cell types within the individual tumors were assessed 8 (42.1%) had heterogeneous tumor cell populations with epithelioid areas showing loss of one copy of chromosome 3 and spindle areas containing 2 copies. The remaining 4 cases (21%) displayed loss of chromosome 3 in both spindle and epithelioid cell areas. The remaining 7 cases of metastasizing and 3 cases of non-metastasizing melanoma contained 2 copies of chromosome 3 in both spindle and epithelioid cells areas.

**Conclusion** Chromosome in situ hybridization is a useful technique for analyzing different cell types within a tumor. In some cases monosomy 3 is correlated with epithelioid cells. Emergence of these monosomic epithelioid subclones within choroidal melanoma may have a role in tumor progression.

■ 4354

## Transscleral biopsy for the determination of the chromosome 3 status in uveal melanomas

*AKGUEL H (1), ZESCHNIGK M (2), SCHILLING H (2), SCHUELER A (2), BORNFELD N (2), ANASTASSIOU G (2)*
*(1) department of ophthalmology, university of Essen/Germany, Essen*
*(2) department of human genetics, university of Essen/Germany, Essen*

**Purpose** Loss of an entire chromosome 3 (monosomy 3) is a frequent chromosomal aberration in uveal melanoma and is a strong predictor for poor prognosis. So far the evaluation of the chromosome 3 status in the tumor is limited to those patients who are treated either by enucleation or tumor resection. However, the majority of patients with uveal melanoma are treated by radiation. For those patients no established method for detecting monosomy 3 is available. In the present study various transscleral biopsy techniques were established. The obtained tumor samples were then tested for their homogeneity by microsatellite analysis

**Methods** Several techniques for transscleral biopsy were investigated on fresh enucleated eyes harbouring uveal melanomas. Chromosome 3 status of the biopsy samples was determined by microsatellite analysis using established chromosome 3 markers. The results were compared to the results from corresponding tumor specimens, which were obtained after opening of the eye globe. Cytology of the biopsy specimens and histogical evaluation of the biopsy sites were performed whenever possible

**Results** By analysing DNA prepared from transscleral biopsy it was possible to determine the chromosome 3 status of a uveal melanoma. The use of a technique based on aspiration was successful but there was a very poor control upon the amount of obtained material thus limiting the use of this method for analysis of thick tumors. An alternative method using special forceps for tissue sampling enables a better control but gains less material

**Conclusion** The appropriate transscleral biopsy technique with a high diagnostic accuracy for monosomy 3 and a safe application is still subject of further research

■ **4355**

## Biopsies for intraocular tumors: the Leiden experience

*MISSOTTEN GS, SWART W, DE KEIZER RJW, DE WOLFF-ROUENDAAL D*
*Ophthalmology, Leiden*

**Purpose** To describe the indications, technique, diagnosis and different staining used, complications and consequences of needle biopsies in intraocular tumors at Leiden University Medical Center (The Netherlands).

**Methods** Patients charts of 5 patients with a choroidal tumor and 10 patients with a intravitreal tumor were reviewed to evaluate the difficulties and pitfalls in the use of biopsies for intraocular tumors

**Results** Although a sample could be taken in all cases, this could not always lead to a definitive diagnosis. Technique, diagnosis and treatment will be reviewed and discussed.

**Conclusion** Needle biopsies remain difficult to procure and to evaluate.

■ **4356**

## Teratoid medulloepithelioma: a case report and literature review

*HADJISTILIANOU T (1), DE FRANCESCO S (1), PIOZZI E (2), CAPOROSSI A (1), CERASE A (3)*
*(1) Ophthalmology, Siena*
*(2) Ophthalmology, Milano*
*(3) Dept. Neuroradiology, Siena*

**Purpose** To describe a case of teratoid medulloepithelioma (TME) in a 1 year-old girl who presented with an pinkish-white mass in anterior chamber.

**Methods** Case report.

**Results** An otherwise healthy baby girl was diagnosed with a rapidly growing, vascularized mass in the anterior chamber of the right eye. A and B ultrasound and MRI have been performed. Differential diagnosis included juvenile xantogranuloma, iris leiomyoma and medulloepithelioma. The baby underwent diagnostic biopsy followed by enucleation. Histopathology revealed a teratoid medulloepithelioma.

**Conclusion** TME is a rare intraocular neoplasm occurring in youg children; the salient clinical and histopatologic features of this rare condition are reviewed.

■ **4357**

## Primary basal cell carcinoma of the caruncle with seeding to the conjunctiva

*HEEGAARD S (1), ØSTERGAARD J (1), PRAUSE JU (1, 2)*
*(1) Eye Pathology Institute, University of Copenhagen, Copenhagen*
*(2) Eye Department, Rigshospitatet, Copenhagen*

**Purpose** To report the clinical and histopathological characteristics of a patient with a primary basal cell carcinoma (BCC) of the caruncle with seeding of the tumour to the conjunctiva.

**Methods** Surgical excision and histological examination.

**Results** A-60-year old female presented with a lesion of the caruncle. Clinical examination revealed a pale lobulated tumour without skin involvement. Computer tomography scans showed orbital invasion. The tumour was excised. Three years later a small polypoid tumour developed in the inferior fornix of the same eye. Two and a half years later the patient developed an orbital recurrence. Microscopically both neoplasms were composed of infiltrative islands of basaloid tumour cells, scattered mitoses and peripheral palisading consistent with the diagnosis of BCC.

**Conclusion** This case describes a primary BCC of the caruncle with seeding to the conjunctiva.

■ 4361

## Inhibition of newly developing blood and lymphatic vessels after high-risk keratoplasty promotes corneal graft survival

*BACHMANN BO (1), LÜTJEN-DRECOLL E (1), WIEGAND S (2), BOCK F (3), STREILEIN JW (4), KRUSE FE (3), CURSIEFEN C (3, 4)*
*(1) Department of Anatomy II, Erlangen*
*(2) Regeneron Pharmaceuticals Inc., Tarrytown*
*(3) Department of Ophthalmology, Erlangen*
*(4) Schepens Eye Research Institute, Boston*

**Purpose** Angiogenesis occurring after low-risk keratoplasty has been identified as a strong risk factor for posttransplantational immune rejections. Aim of this study was to evaluate whether after high-risk keratoplasty additional hem- and lymphangiogenesis occur and whether inhibition of this additional vessel ingrowth also promotes corneal graft survival.

**Methods** Three interrupted 11-0 sutures were placed into the corneal stroma of BALB/c mice and left in place for 6 weeks. Three weeks after suture removal penetrating keratoplasty was performed with same-aged C57BL/6 donors in the mouse model of corneal transplantation. Mice in the treatment group received a VEGF-A specific cytokine trap (VEGF TrapR1R2) intraperitoneally at the day of surgery as well as 4, 7 and 14 days later (25 mg/kg/mouse; n=13). Control mice received the same amount of Fc-protein at the same timepoints (n=10). Postoperative survival of the grafts was analyzed for 8 weeks using a slit-lamp and Kaplan-Meier survival curves. Morphometrical quantification in corneal wholemount preparations was performed using the software ImageJ.

**Results** After high-risk keratoplasty an additional ingrowth of blood (30%) and lymphatic (33%) vessels compared to the situation directly prior to transplantation occurred. This additional ingrowth was significantly inhibited under treatment with the VEGF Trap. All control corneas were rejected after 3 weeks, whereas 23% (3) of the treated corneas were still un-rejected after 8 weeks($p<0.05$).

**Conclusion** These data support the new concept that surgery-related hem- and lymphangiogenesis after high-risk keratoplasty contribute to the development of immune rejections after grafting.

■ 4362

## PI-88 inhibits infectivity and cell-to-cell spread of HSV-1 and -2 and displays antiinflammatory activity

*FERRO V (1), TRYBALA E (2), BERGSTRÖM T (2), PARISH C (3), FREEMAN C (3), CHAPMAN P (4)*
*(1) Progen Industries Ltd, Brisbane*
*(2) Clinical Virology, Göteborg*
*(3) Immunology & Genetics, John Curtin School of Medical Research, Canberra*
*(4) OE Ireland Ltd, Dublin*

**Purpose** Herpes simplex virus (HSV) infections of the eye manifest as keratoconjunctivitis with associated painful dendritic lesions. Topical treatments with antiviral agents are largely ineffective in shortening the symptoms or the duration of the primary attack (even when used in combination with steroids) and in preventing recurrent attacks in susceptible individuals. PI-88 may be a novel and useful treatment for ocular HSV because it combines inhibition of infectivity and cell-to-cell spread with antiinflammatory activity.

**Methods** In vitro viral plaque assays and animal models of inflammation.

**Results** PI-88 is a non-cytotoxic heparan sulfate (HS) mimetic that inhibits infection and spread of HSV by interfering with the binding of HS to viral envelope glycoproteins. Inhibition of HSV spread is not seen with larger sulfated polysaccharides and is probably due to PI-88's ability to access the narrow intercellular space. PI-88 also displays antiinflammatory activity in animal models mediated by its inhibition of heparanase, a key enzyme involved in the extravasation of leucocytes to sites of inflammation.

**Conclusion** The combination of potent inhibition of HSV infectivity and spread with antiinflammatory activity makes PI-88 a potentially useful treatment for ocular HSV lesions. This offers the possibility of a topical antiiflammatory and antiinfective monotherapy to prevent reoccurrence of HSV infection which is still a significant cause of blindness. [Other clinical uses of Pl-88 by topical ocular therapy are being investigated where its HS mimetic activity on cell surface receptors may moderate ocular surface disease activity or immune response].

■ 4363

## Epidemiology of uveitis : a study of 634 patients from a single tertiary center over a period of 1 year

*BODAGHI B (1), TERRADA C (1), MIYARA M (2), CASSOUX N (1), WECHSLER B (2), LE THI HUONG D (2), ROZENBERG O (1), FARDEAU C (1), PIETTE JC (2), LEHOANG P (1)*
*(1) Ophthalmology, Paris*
*(2) Internal Medicine, Paris*

**Purpose** To evaluate the clinical features, the etiological distribution and the therapeutic management of patients referred to a tertiary eye care center.

**Methods** Clinical findings, etiology and treatment of uveitis of all patients referred for the first time between January 2001 and January were retrospectively analyzed.

**Results** A total of 634 patients were included (Mean age : 40.4±19.1 years). Male to female sex-ratio was 0.82. Acute uveitis (less than three months of evolution) represented 35% of cases. Uveitis was unilateral in 51% of cases and severe in 19.4% of cases. Posterior uveitis was the most frequent anatomical form (33.3%) followed by anterior (26.8%), panuveitis (23.7%), intermediate (11.8%) and kerato-uveitis (4.4%). An etiological diagnosis was established in 65.1 % of cases. It was suspected during the first visit in 76 % of those cases. Leading causes of uveitis were infections (28%) especially toxoplasmosis (10.6%) and herpes infections (5.7%), then inflammatory diseases (17.7%) including sarcoidosis (5%) and Behçet disease (2.8%). HLA-B27-linked uveitis was under–represented (5%) since its diagnosis and management rarely require a tertiary eye care center. Most of patients (54.7%) were immediately treated with systemic anti-infectious (antiviral, anti-parasitic, antibacterial) therapies (48.4% of treated patients) or systemic steroids and/or immunosuppressive regiments (42.1% of treated patients).

**Conclusion** Great diversity of causes, importance of accurate etiological diagnosis for adapted treatments and management of systemic therapies emphasize the collaboration between ophthalmologists, rheumatologists and internists.

■ 4364

## Klebsiella Endogenous Endophthalmitis- An Asian Perspective

*CHENG BCL, CHEE SP*
*Singapore National Eye Centre, Singapore*

**Purpose** To analyse the risk factors, clinical presentation and prognosis of Klebsiella Endogenous Endophthalmitis (KEE) in Singapore

**Methods** Retrospective, non-comparative case series looking at culture proven (KEE) cases seen in the Singapore National Eye Centre from 1986 - 2003.

**Results** A total of 40 patients with 46 eyes, male to female ratio 28:12, mean age 54.3 (25 – 81) were included. Hepatobiliary tract infection (65%)represented the most common source of infection, followed by Urinary tract (12.5%), chest (7.5%),and others (15%). Hypopyon with posterior diffuse intraocular inflammation was the commonest presentation(72%). Majority (85%) presented with counting fingers or worst visual acuity. Diabetes mellitus was a significant risk factor present in 45% of the patients. 95.6% had positive blood cultures as compared to 56% of positive vitreous cultures. The use of intravitreal antibiotics and vitrectomy did not significantly affect visual outcome. 63% of patients had a final visual acuity of no perception of light.

**Conclusion** KEE is a devastating ocular complication of systemic infection with hepatobiliary tract sepsis as the most common source. Diabetic mellitus is an important risk factor (45%). Majority had no perception of light despite intravitreal antibiotics and vitrectomy.

■ **4365 / 478**

## Complications of Pars Plana Vitrectomy in Acute Endophthalmitis

*GARCHER CREUZOT CP (1), LAFONTAINE PO (1), PASSEMARD M (1), ROUBEROL F (1), CORNUT PL (2), BRON AM (1), THURET G (3), ROMANET JP (4), CHIQUET C (4)*
*(1) Department of Ophthalmology, Dijon*
*(2) Department of Ophthalmology, Lyon*
*(3) Department of Ophthalmology, Saint-Etienne*
*(4) Department of Ophthalmology, Grenoble*

**Purpose** To assess the results and the complications of vitrectomies performed in acute endophthalmitis.

**Methods** Forty–eight patients aged from 22 to 96 years old (69± 16) were included in this multicenter prospective study. All patients presenting an acute postoperative endophthalmitis and undergoing a vitrectomy as part of their treatment were enrolled.

**Results** Visual acuities were light perception (50%), hand motion (35.5%) or between 20/400 to 20/200 (14.5%) before vitrectomy. Vitrectomy was performed with a mean delay of 5.2 days after the onset of the hospitalization. Per operative complications were uncommon: vitreous IOL luxation (n=1), intra vitreous hemorrhages (n=2). Postoperative complications were phthisis (6%), retinal detachment (10.5%), irido–capsular synechiae (12.5%), epiretinal membrane (8%) and ocular hypertension (14.5%). Final visual acuities were 20/20 (8,5%), from 20/40 to 20/25 (21%), from 20/200 to 20/50 (31.5%), count fingers (16.5%), hand motion (6%), light perception (6%) and no light perception (10.5%).

**Conclusion** Vitrectomy performed as a treatment modality for endophthalmitis remains a difficult vitreous surgery due to the poor visibility of the posterior segment (cornea edema, anterior segment's flare, vitreous opacities). The most frequent complications are similar to vitreous surgeries done in other indications than endophthalmitis i.e. retinal detachment, epiretinal membrane and ocular hypertension. Final visual impairment or blindness is mainly due to phthisis or untractable infection rather than vitrectomy.

■ **4366 / 479**

## Comparative study of acute post-operative endophthalmitis with or without microbiological identification

*VINH V (1), CORNUT PL (2), BENITO Y (3), LAFONTAINE PO (4), THURET G (5), ROUBEROL F (2), BRON AM (4), DENIS P (2), CREUZOT-GARCHER C (4), VANDENESCH F (3), ROMANET JP (1), CHIQUET C (1)*
*(1) Ophthalmology, Grenoble*
*(2) Ophthalmology, Lyon*
*(3) MICROBIOLOGY, Lyon*
*(4) Ophthalmology, Dijon*
*(5) Ophthalmology, Saint-Etienne*

**Purpose** To compare epidemiological and clinical data of patients with acute post operative endophthalmitis with microbiological positive ocular samples and those with negative ocular samples.

**Methods** This multicenter prospective study includes 87 patients hospitalized for a post operative endophthalmitis between 2002 and 2005, as a complication of cataract surgery (n = 77), filtering surgery (n = 6), vitreoretinal surgery (n = 2), strabismus surgery (n = 1) or a radial keratotomy (n = 1). A bacteria was identified in aqueous humour and/or in vitreous, by conventional cultures and polymerase chain reaction (PCR) for 69% patients.

**Results** Microbiological proven endophthalmitis differs from sterile endophtalmitis (reduced delay of onset, higher intraocular pressure, rate of vitrectomy and initial and final visual acuity). Patients with a microbiological proven endophthalmitis were not different from patients with negative cultures and PCR for the following criteria : age of patient, type of the initial surgery, functional (pain, visual loss, redness, photophobia, secretions) and clinical signs (corneal edema, hypopion, hyalitis).

**Conclusion** Using cultures and PCR on ocular samples, the positivity of the microbiological identification is associated with some clinical signs. These findings are partly consistent with previous results of the Endophthalmitis Study group (association of no growth with time from surgery to symptoms, hypopion, initial and final visual acuity, media clarity). However the patient's initial examination is not highly predictive of the results of cultures and PCR.

## ▪ 4411
## Transmission – acquired X congenital and new aspects

*STANFORD M*
*Medical Eye Unit, St Thomas' Hospital, London*

**Purpose** Infection with Toxoplasma gondii is common and ocular disease is the commonest clinical manifestation of infection. Based on serological studies at least one third of the world's population has been infected. Infection occurs through ingestion of the parasite in poorly prepared foodstuffs or from drinking contaminated ground water. Vertical transmission of the parasite from mother to foetus occurs and may result in congenital toxoplasmosis, the principal component of which is retinochoroiditis. Certain countries such as those in West Africa and Brazil show an extremely high prevalence and in these countries ocular disease resulting from infection is common. Whilst infection rates are highest in children, the commonest time for presentation with symptomatic eye disease is around 30 years of age.Previous dogma held that the majority of ocular disease was due to the late onset of congenitally acquired infection. However, epidemiological evidence has refuted this view and it is now estimated that more than 60% of ocular disease seen in European countries is a result of post-natally acquired infection. Recent work on the presentation of symptomatic retinochoroiditis in children shows that one half of disease is due to congenital and one half to acquired infection, the former presenting before the age of three and the latter after ten years of age. The implications for this on pre- and neonatal screening programs, particularly in the absence of any evidence that treatment is effective, needs further assessment.

## ▪ 4412
## Neonatal Toxoplasmosis – long-term follow-up

*KODJIKIAN L*
*Croix-Rousse Hospital, Lyon*

**Purpose** Retinochoroiditis is the most common ocular manifestation of congenital toxoplasmosis, but other associated ophthalmological pathologies can also occur. The aim of this study was to determine the nature of the latter in treated cases of the disease and to assess their impact on visual function.

**Methods** Four hundred and thirty consecutive children with serologically confirmed congenital toxoplasmosis were included in this study. Data were prospectively collected using standardized ophthalmological assessment forms. The presence of retinochoroiditis and of associated pathologies was ascertained, and their impact on visual function was assessed.

**Results** After a median follow-up of 12 years [range 0.6-26 years], 130 children manifested retinochoroiditis. Twenty-five of the 130 children (19%) had other associated ocular pathologies. Of these, 21 (16%) had a strabismus, which was due to macular lesions in 86% of the cases; 7 (5.4%) presented with unilateral microphthalmia, and 4 (3%) with cataracts. Macular lesions occurred more frequently in children with associated pathologies ($p<0.0001$), and associated pathologies were likewise more common in individuals with macular lesions ($p=0.0003$).

**Conclusion** At the end of the follow-up period, ocular involvement existed in 30% of the treated children with congenital toxoplasmosis. Associated eye pathologies were manifested less frequently than anticipated. They may occur later in life and are an indirect marker of the severity of congenital toxoplasmosis, but they do not have a direct impact on visual acuity. The overall functional prognosis of congenital toxoplasmosis is better than would be expected on the basis of literature findings, with only 2 of the 130 children suffering bilateral visual impairment.

## ▪ 4413
## Clinical Picture – general considerations

*PAVESIO C*
*Moorfields Eye Hospital - Medical Retina, London*

**Purpose** Ocular toxoplasmosis is the most common form of posterior uveitis in most countries. The classic presentation is of a focal necrotizing retinitis, generally associated with vitritis and, in many cases, anterior uveitis, which is typically hypertensive. This inflammatory process is usually sef-limited in the immunocompetent host. Lesions involving the posterior pole will produce a significant change in vision while the more peripheral ones will produce symptoms if associated with intense vitritis. The ocular manifestations also include involvement of the retinal vessels in the form of vascular sheathing and vascular occlusions. Lesions occurring in the vicinity of the optic disc may result in loss of central vision and/or visual field. Visual loss may occur as a consequence of direct macular involvement by the active focus of infection or by the development of a choroidal neovascular membrane; it may also be the result of optic neuropathy, vitreous opacity or retinal detachment.In the immunocompromised individual the clinical presentation is that of multiple or bilateral foci of infection, with a tendency to progression if not treated.Therapeutic strategies in the immunocompetent, immunocompromised and in the setting of pregnancey will be discussed.

## ▪ 4414
## Diagnostic Considerations

*PLEYER U, TORUN N*
*Charite, Berlin*

**Purpose** Ocular toxoplasmosis (OT) remains a major cause of posterior uveitis. The diagnosis of OT in immunocompetent adults is usually made by ophthalmoscopic detection of a solitary focus of active chorioretinitis adjacent to a chorioretinal scar. Although the ophthalmologic signs of OT are highly suggestive, they can be mimicked by other infections or the clinical presentation can be atypical. Therefore laboratory testing may become crucial. Since seropositivity is only indicative of infection, analysis of intraocular fluids and tissues has become an important implication for diagnosis.

**Methods** In recent years several diagnostic approaches have been studied. Histopathologic proof of the organims is the gold standard, but ocular biopsies in OT are only rarely justified. Direct isolation of T. organisms from ocular fluid has been reported in tissue culture systems developed for viral culture. However, results take on average 12 days and need to be confirmed by microscopy, fluorescent antibody staining, and PCR.In contrast, PCR of T. DNA is more rapid than culture and requires only a small sample. However the DNA content in aqueous humor is low and results in an relatively high number of false negatives. Detection of specific antibodies against T. in ocular fluids is an alternative strategy that complements PCR testing. Interestingly, both timing and immune status probably influence results of antibody testing. Whether a delay in the onset of local specific antibody production reflects immune tolerance in patients with congenital OT remains unclear, but it may account for a relatively low confirmation rate for local antibody production.

**Conclusion** Taken together, current diagnostic strategies for OT need to be re-evaluated in order to improve the diagnosis of this common and sight threatening infection.

■ **4415**

## Immunology of Toxoplasmosis

*LIESENFELD O*
*Inst. for Infection Medicine, Dept of Medical Microbiology and Immunology of Infection, Charité Medical School Berlin, Berlin*

**Purpose** Infection with T gondii results in a strong and persistent T-helper-1 (Th1) response characterized by production of pro-inflammatory cytokines including IL-12, IFN-γ, and TNF-α. The combined action of these cytokines and other immunological mechanisms protects the host against rapid replication of tachyzoites and subsequent pathological changes. After invasion of enterocytes, T gondii infects APCs in the intestinal lamina propria and induces a local Th1 response. Dendritic cells are the main activators of the Th1 immune response after infection of mice with T gondii. Granulocytes can also contribute to early production of IL-12. The activated macrophage inhibits or kills intracellular T gondii. However, T gondii exploits APCs as "Trojan horses" by down-regulation of cell-surface molecules and interference with apoptosis pathways. Sensitized CD4+ and CD8+ T lymphocytes are both cytotoxic for T gondii-infected cells. Pro-inflammatory (IFN-γ, TNF-α) and downregulatory (IL-10, TGF-β) cytokines are both involved in balancing of this response. The proportion of γ/δ T cells is enhanced during acute infection. Within 2 weeks after infection, IgG, IgM, IgA, and IgE antibodies against many T gondii proteins can be detected. Production of IgA antibodies on mucosal surfaces seems to protect the host against re-infection. Eye infection in immunocompetent patients produces acute chorioretinitis characterized by severe inflammation and necrosis. The pathogenesis of recurrent chorioretinitis is controversial. Rupture of cysts can release viable organisms that induce necrosis and inflammation; alternatively, chorioretinitis can result from a hypersensitivity reaction triggered by unknown causes. In light of these findings, results from studies in experimental models of eye infection will be discussed.

■ **4416**

## New findings : possible role of Toxoplasma gondii in the developement of primary intraocular lymphoma

*HERBORT CP (1, 2), PERENTES Y (1), CHAN CC (3)*
*(1) Inflammatory Eye Diseases, La Source Eye Centre, Lausanne*
*(2) University of Lausanne, Lausanne*
*(3) Laboratory of Immunology, NEI, Bethesda*

**Purpose** Primary intraocular lymphoma (PIOL) can occur alone or in association with CNS involvement. The vast majority of PIOLs are diffuse large B-cell lymphomas (DLBCL). Two molecularly distinct forms of DLCBLs have been identified one of which, « activated B-like DLBCL», is thought to be antigen driven. Toxoplasma Gondii (T.Gondii), a protozoan parasite, is the most common cause of posterior uveitis in Europe. We report here the association of PIOL with severe posterior toxoplasmosis.

**Methods** Included were all patients with posterior toxoplasmosis seen at our center with a resistent course and for whom a diagnostic vitrectomy was decided. Vitrectomy specimen were sent to the Laboratory of Immunology, NEI, Bethesda, MD, where microdissection of cells and PCR detection of the rearranged FR3 gene and bcl-2 gene translocation, as well as PCR detection of T.gondii genes (B1).

**Results** From 1995 to 2003, 72 of 742 uveitis patients (9.7%) were diagnosed as posterior toxoplasmic. 4/72 (5.5%) had a protracted/recurrent form of disease and vitrectomy was performed in 3. In 2/3 patients PIOL was diagnosed. FR3 gene rearrangement was present in both and bcl-2 gene translocation was present in one. In 1/3 patients T. gondii DNA detected by PCR was found in the vitreous. In 1/2 patients with lymphoma cells, T.gondii genoma (B1 gene) but not EBV nor HHV-8 genes was detected within the DNA of the malignant cell.

**Conclusion** This is the first report of PIOL found in association with severe forms of toxoplasmic retinochoroiditis and of incorporation of parasitic DNA into neoplastic cells. T.Gondii may thus play a role in lymphomagenesis and antigen driven PIOL may be a subgroup in the heterogeneous group of diffuse large B-cell lymphoma (DLBCL)

■ 4421

## 4 years ELT in Detmold: experiences & results

*KLEINEBERG L*
*Augenklinik Detmold, Detmold*

**Purpose** In the late nineties ELT was expected to become the new ultimate glaucoma surgery procedure. After first rather disappointing experiences most ophthalmic surgeons lost their interest. Apparently there is a certain "learning curve" which requires some time to achieve reproducible results. Only few ophthalmological centers continued to perform ELT

**Methods** Our clinic continued with ELT. Since september 2001 we have carried out more than 200 ELT's and monitored further progress. 100 Patients were included to our study. the study is a cooperation with the University of Dresden. We formed 3 groups for the study. In groups I and II ELT was made as standalone procedure, in gruop III additionally to phacoemulsification. Groups I and II received different postoperative medication.

**Results** After 24 months we can show that IOD-lowering effect has been significant in all groups. Average IOP-lowering was 34%. Additionally the giving of antiglaucomatous medicaments could be cut down by 1.3 per patient.

**Conclusion** ELT shows significant IOD-lowering effect and bears minimal risks. So it's a convincing and convenient alternative to filtrating surgery.

■ 4422

## Excimer Laser Trabeculotomy (ELT) in Freiburg– two years follow up

*WILMSMEYER S*
*Freiburg*

**Purpose** To evaluate the efficacy and safety of endoscopic laser trabeculotomy in combination with or without cataract surgery in patients with ocular hypertension or glaucoma.

**Methods** An endoscope guided photoablative laser (Excimer laser, AIDA, TUI-Laser, Munich) was used to induce microperforations in the trabecular meshwork. Altogether n=135 eyes were enrolled in our retrospective study (n=75 with ELT, n=60 with ELT and cataract surgery). Intraocular pressure (IOP) and the number of antiglaucoma drugs (AGD) were recorded preoperatively, 2-4 mths (T1), 5-7 mths (T2), 11-13 mths (T3) and 22-26 mths (T4) after the surgery. Kaplan Meier analysis was used to calculate the percentage of successful treatments. A treatment was defined to be successful if I) postoperative IOP was below or equal to 21 mm Hg, and II) IOP reduction was at least 20%.

**Results** ELT reduced the IOP from 24.1±0.7 mmHg preoperatively to 18.8±0.4 (T1), 20,0±0.5 (T2), 18.8±0.8 (T3), 16.8±1.0 (T4) mmHg respectively. The number of AGD was 1.9±0.1 (T0), 1.2±0.2 (T1), 1.3±0.2 (T2), 1.8±0.2 (T3) and 1.5±0.3 (T4). The success rate of ELT alone was 60 (T1), 49 (T2) and 46 % (T3).Combined phacoemulsification + ELT reduced the IOP from 22.4 mmHg±0.6 (T0) to 16.5±0.4 (T1), 16.1±0.5 (T2), 16.4±0.4 (T3),12.8±1.5 (T4) mmHg respectively. The number of AGD was 1.1±0.2 (T0), 0.9±0.2 (T1), 1.1±0.2 (T2), 1.3±0.2 (T3) and 1.8±0.9 (T4). The success rate was 85 (T1), 74 (T2) and 66 % (T3).

**Conclusion** ELT, especially in combination with phacoemulsification, is a new promising minimally invasive lasertreatment to reduce intraocular pressure.

■ 4423

## Does the success rate of ELT depend on preoperative IOP?

*LAUTEBACH S, WILMSMEYER S, PACHE M, FUNK J*
*Freiburg*

**Purpose** Excimer-Laser-Trabeculotomy (ELT) is a new, minimally invasive surgical procedure for the treatment of open-angle glaucoma .The ELT can be performed alone or in combination with with cataract surgery. We herein report our experience with ELT as a stand-alone procedure and ELT plus cataract surgery.

**Methods** A total of 135 patients with open-angle glaucoma (n = 128) or ocular hypertension (n = 7) (M:F = 49:86, mean age 69,7 ± 1,2 years) were included in this study. Patients were divided into 2 groups: a) ELT as a stand-alone procedure (n=75), b) combined cataract and ELT procedure (n= 60). Both groups were further divided into 2 subgroups: 1. Preoperative IOP > 22mmHg, 2. Preoperative IOP ≤ 22mmHg. Success criterion was 20% decrease of IOP and IOP ≤ 21 mmHg and postoperative IOP-lowering medication ≤ preoperative IOP-lowering medication. Kaplan-Meier survival curves were calculated for a one year follow-up time.

**Results** Group a) ELT, 1. Preoperative IOP > 22mmHg: Kaplan-Meier survival curves showed a success rate of 57%, 2. Preoperative IOP ≤ 22mmHg: success rate was 41% after one year. Group b) Combined cataract and ELT procedure, 1. Preoperative IOP > 22mmHg: success rate was 91%, 2. Preoperative IOP ≤ 22mmHg: success rate was 52%.

**Conclusion** ELT is a promising IOP-lowering technique as a stand-alone procedure and especially in combination with cataract surgery. The results are depending on the preoperative IOP. Subjects with higher preoperative IOP showed better survival curves.

■ 4424

## Prospective Investigation of ELT

*PACHE M*
*University Eye Clinic, Freiburg*

**Purpose** Presentation of a study design to determine the safety and efficacy of excimer laser trabeculotomy (ELT) in reducing intraocular pressure (IOP) in patients with primary open angle glaucoma with (PIE+) and without (PIE) concurrent cataract surgery.

**Methods** The University Eye Clinic Freiburg coordinates two prospective, non-randomized, consecutive, open labeled, multi-center, clinical evaluation studies. We plan to conduct the studies at multiple investigational sites. Each site will enroll subjects for a total of 53 subjects for each study in order to obtain safety and efficacy data for ELT, using the AIDA Laser excimer laser system. In the PIE Study, a small clear corneal incision is prepared and an endoscope is placed in the anterior chamber. The ELT procedure involves application of 10 openings in the inferior trabecular meshwork under optical control. In the PIE+Study, cataract surgery is performed through a clear cornea tunnel incision. After phacoemulsification of the lens, a foldable intraocular lens is inserted. The same clear cornea incision used to perform cataract surgery is used for ELT. Primary effectiveness measure is IOP as measured by Goldmann applanation tonometry. Secondary effectiveness measure is glaucoma drug therapy - number, type and frequency of medications preoperatively versus postoperatively, and, in the PIE+ study, also the effect of the concurrent cataract surgery.

**Results** The studies have been approved by the Ethics committee and have started in 3 centers. Results are not yet available. Further centers who like to join the study are welcome.

**Conclusion** Prospective results about safety and efficacy of ELT and combined ELT+ Cataract surgery can be expected in the near future.

■ 4425

## ELT & Blue blood

*GIERS UF, KLEINEBERG L*
*Augenklinik, Detmold*

**Purpose** Couple of years ago excimer laser trabeculostomy was expected to become a new ultimate method of glaucoma surgery. After first rather disappointing experiences most surgeons lost their interest.

**Methods** Apparently there is a certain learning curve which allows surgeon achieve reproducible results. So only few ophthalmological centers (including us) continued performing ELT.This video demonstrates the effectiveness of ELT 18-24 months postoperatively in 2 cases. App. 2 years after successful ELT we performed cataract surgery on the same eye.

**Results** Prior to phacoemulsification anterior chamber was filled with air and Vision Blue. After 30 seconds we observed blue episcleral veins in nasal inferior quadrant - the quadrant with ELT-effects. Episcleral veins in other quadrants remained unchanged.

**Conclusion** This should prove that excimer laser trabeculostomy ab interno is a convincing and convenient alternative to filtrating surgery.

■ 4426

## Excimer Laser Trabeculotomy (ELT) - Clinical Update

*FUNK J*
*Universitaets-Augenklinik, Freiburg*

**Purpose** Trabecular meshwork photoablation was introduced to glaucoma surgery in 1996 by Vogel et al. (Ophthalmologe 93:565). Several prototypes of lasers were used since this time. Meanwhile, a CE-certified device is commercially available. The procedure now is usually called Excimerlasertrabeculotomy (ELT)

**Methods** ELT uses photoablation to create several holes within the trabecular meshwork, thus improving aequoes outflow. No thermal necrosis is seen in the adjacent tissue. The operation is monitored by a goniolens or by an endoscope.

**Results** The IOP lowering effect of ELT ranges from 20% to 40%, depending on the preoperative value and whether it is combined with cataract surgery. Complications are extremely rare. IOP reduction remains stable for at least one to two years (this is the longest available follow up). Former studies with comparable devices (Er:YAG goniopuncture) showed that the effect of trabecular meshwork photoablation presumably will even hold on for 5 years or more.

**Conclusion** Trabecular photoablation ab interno is a promising new kind of minimally invasive glaucoma surgery. Further improvement regarding the endoscopic monitoring or the postoperative treatment still might enhance its success rates

## ▪ 4431
## Methods to measure MP

*VAN NORREN D (1), BERENDSCHOT TTJM (2)*
*(1) Ophthalmology, UMC Utrecht, Utrecht*
*(2) University Eye Clinic Maastricht, Maastricht*

**Purpose** To present an overview of the methods to assess the optical density of macular pigment (MP) in the living human eye.

**Methods** The relevant literature was reviewed.

**Results** Methods fall in two main classes: Those using the human eye as a detector (psychophysics) and those using a physical device as a detector. The most used psychophysical method (heterochromatic flicker photometry, HFP) requires the subject to minimize flicker at the fixation point, and at a peripheral location where MP is supposed to be absent. HFP is reliable but time consuming. The physical devices are based on three approaches: Fundusreflectometry, autofluorescence, and resonance Raman spectometry. Devices are generally custom build and costly, but fast and reproducible results may be obtained, even without pupil dilatation. All methods have their specific advantages and disadvantages, but Raman spectometry still meets substantial debate. Correlations between outcomes of different methods range from medium to high.

**Conclusion** A golden standard in measuring macular pigment does not exist. HFP is most widespread, but a physical method might win as soon as a low to medium cost apparatus becomes available. Recent emphasis on the importance of the retinal distribution of macular pigment complicates matters.

## ▪ 4432
## The Relation of Macular Pigment Optical Density and Plasma Levels of Lutein and Zeaxanthin to their Dietary or Supplementary Intake – an Overview of Human Studies.

*SCHALCH W*
*R&D Department, DSM Nutritional Products Ltd., Kaiseraugst*

**Purpose** Lutein (L) and Zeaxanthin (Z) are two xanthophylls that naturally accumulate in the macula lutea, where they form the highest concentration of carotenoids found in the human body. While plants can bio-synthesize carotenoids, the human body has to rely on external sources for their supply. This talk will review data from human studies that have evaluated how dietary and supplementary intake of L and Z relate to their plasma concentrations and to macular pigment optical density (MPOD).

**Methods** The reviewed studies have measured plasma levels of carotenoids by HPLC. MPOD was determined by a number of techniques. Dietary intake was assessed by questionnaires. L was isolated from marigold petals and Z (OPTISHARPTM) chemically synthesized.

**Results** It is generally accepted that dietary or supplementary intake of L or Z leads to measurable increases of MPOD. In few cases of supplementation, however, no increase is measurable. Recent data indicate that upon supplementation the xanthophylls may not only accumulate in the central retina (fovea) but also in the parafovea. If this is true, it suggests that techniques that rely on a parafoveal reference location for MPOD measurements should be modified if to be used in supplementation studies.

**Conclusion** The intake of L and Z, either through dietary intake or by supplementation, leads to increases of plasma levels and MPOD. The response is quite variable and MPOD responses may not be measurable in all subjects. Whether this is due to the existence of MPOD responder and non-responder subjects or to parafoveal pigment accumulation upon supplementation should be one of the topics of future research.

## ▪ 4433
## MP and its relation with AMD

*DAVIES N*
*Ophthalmology, Chelsea and Westminster Hospital, London*

**Purpose** To examine the relationship between the macular pigments (MP) and age-related macular degeneration (AMD)

**Methods** Review of current literature pertinent to macular pigments and the role they play in protecting the macula from AMD

**Results** The MP form part of the anatomical specialization of the macula. MP have been located in the inner plexiform layer and the photoreceptor axons. They have also been found in rod outer segments.There are two clearly established properties of the MP: they absorb blue light and they act as scavengers of free radicals. AMD is the commonest form of blindness in the Western world and its incidence is likely to increase as longevity increases. The pathogenesis of AMD is multifactorial and not fully understood but there is good evidence to support oxidative stress as one of the mechanisms. Oxidative stress in the macula results from absorption of high energy photons and from biochemical processes in an environment of high oxygen tension. The presence of MP may therefore protect the macula from damage by both known properties.There is some evidence to support the hypothesis that MP can reduce risk of AMD. Serum levels of MP were lower in those with AMD than those without. Risk factors for AMD include cigarette smoking, obesity, light iris color and female gender. All of these have also been shown to be independently associated with lower than average macular pigment optical density (MPOD). It has been shown that MPOD can be elevated by dietary supplement. Trials of dietary supplement are underway to assess the effect of higher MP levels in the macula with risk of developing AMD.

**Conclusion** There is associative evidence that MP protect the macula from AMD. The results of oral supplement studies are awaited with great interest.

## ▪ 4434
## Macular pigment: what do we know about the spatial distribution?

*TRIESCHMANN MB, PAULEIKHOFF D*
*Department of ophthalmology St.Franziskus Hospital, Münster*

**Purpose** As Macular pigment (MP) consisting of Lutein(L) and Zeaxanthin (Z) has antioxidative qualities its distribution is of clinical interest. The increase of MP optical density (MPOD) under dietary supplementation could offer a preventive approach to age related macula degeneration (AMD).

**Methods** Histological findings and in vivo measurements of MPOD are presented. In-vivo MPOD determinations are performed by autofluorescence (AF) mapping employing 488nm and 514nm, computerised alignment of both maps and quantitative analysis of grey scale values.This strategy provides density maps of MP distribution and was used a one year supplementation study of L and Z (110 probands ,12 mg L,1 mg Z per day).

**Results** Histological findings showed a variation in the distribution of MP between individuals (range of max. MP from 200-700µm, min MP from 300-900µm radius). MP is mainly located in the layer of Henle, 25% in rod and cone outer segments. Z is dominant in the center of the fovea, L prevails in the periphery. The MP distribution within AF mapping confirms the inter- individual variation. Probands with low levels of MP at 0,5° eccentricity at baseline showed early and good response to supplementation with 12mg L and 1 mg Z , while those with high levels of MP at baseline had late and poorer response.

**Conclusion** Distribution of MP differs inter- individually as shown in histological and in vivo analyses. The role of MPOD pattern in density maps including ring like structures at about 1-1,5 degree eccentricity has to be discussed. Several Maps of MP distribution before and after supplementation can be shown and discussed. Further studies have to investigate the prognostic implication of MP distribution in order to define a specific risk profile.

■ **4435**

## MP and its optical function(s)

*WEALE RA (1, 2)*
*(1) Institute of Gerontology, King's College London, London*
*(2) University College London Hospital Eye Department, London*

**Purpose** To ask whether the macular pigment serves to control chromatic aberration.

**Methods** Some of the relevant work on the subject will be reviewed in an attempt to examine the wider biological context of the subject. A review of some of the work carried out during the last hundred years may well reveal that the correction of (longitudinal) chromatic aberration is not the most important function of the pigment.

**Results** The balance of evidence recently obtained points to the role of the macular pigment playing a smaller role, if any, in the control of chromatic aberration than may have been thought in the past. However, it needs testing with controlled studies on a larger number of observers of different ages.

**Conclusion** Perhaps we ought to forget about chromatic aberration, and start thinking about evolution.

■ 4441

## Hyperoxia-induced retinal dysfunction in rats: consequences on visual responses in the midbrain

*CASANOVA C (1), ZABOURI N (2), HUPPE-GOURGUES F (3), CHEMTOB S (4), LACHAPELLE P (5)*
*(1) School of Optometry, Universite de Montreal, Montreal*
*(2) Neurology & Neurosurgery, McGill, Montreal*
*(3) Physiology, Universite de Montreal, Montreal*
*(4) Pharmacology, Universite de Montreal, Montreal*
*(5) Ophthalmology, McGill, Montreal*

**Purpose** Postnatal hyperoxia causes irreversible structural and functional damage to the retina of newborn Sprague-Dawley (SD) rats. The present study investigates the impact of the hyperoxia-induced retinal dysfunction on responses in the superior colliculus (SC), a prominent direct target of retinal ganglion cells.

**Methods** Newborn SD rats were exposed to hyperoxic conditions as previously described (Dorfman et al., IOVS, 44: 2003). At adulthood, ocular injections of the beta subunit of cholera toxin (CTB) were performed in order to quantify the terminal boutons at the level of the SC. Carbon-fiber electrodes were used to record visual evoked potentials (VEPs) in the colliculus as a function of depth.

**Results** The CTB injections revealed that there was a significant reduction of retinal fibers in some areas of the SC of hyperoxic rats. In addition, areas receiving retinal projections appeared to have a reduced number of terminal boutons despite the fact that they exhibited an absolute density of ganglion endings similar to that of normoxic rats. In control rats, the VEPs included an early (30ms) biphasic potential and a late positive waveform of smaller amplitude (120 msec). In exposed animals, the amplitude of the early waveform was strongly reduced and its latency was increased. Moreover, the late waveform appeared earlier and was almost null at the inversion depth and below.

**Conclusion** These results suggest that the hyperoxia-induced changes observed at the level of the SC, such as the decrease of the short latency waveforms, may be explained in part as a direct consequence of an abnormal retinal function and/or an abnormal retinofugal pathway (as suggested by the reduction of terminal boutons).

■ 4442

## Neural-metabolic coupling in the central visual pathway

*FREEMAN RD*
*Group in Vision Science, Helen Wills Neuroscience Institute, University of California, Berkeley*

**Purpose** In fMRI, the blood oxygen level dependent (BOLD) signal is used to infer neural activity. One of the important limits of this method is spatial resolution. Ideally, the utility of this procedure would be vastly improved if it allowed the monitoring of neural changes at a spatial scale that would permit the resolution of columnar structure within the cerebral cortex. We have undertaken measurements that are intended to elucidate relationships between metabolic and neural functions directly.

**Methods** We have constructed a double barrel micropipette electrode system by which we are able to make simultaneous measurements of tissue oxygen concentrations and single-cell neural activity within the visual cortex. This approach has the advantage of enabling direct measurements of both neural and metabolic activity in a limited volume of co-localized cortical tissue.

**Results** Our findings are as follows. As neural activity is raised by appropriate visual stimulation, there is an immediate proportional decrease in tissue oxygenation indicating a close link between the two functions. To pursue this finding, we used the decrease in tissue oxygenation to predict two unique functional properties of visual cortical cells in neighboring neurons: orientation selectivity and ocular dominance

**Conclusion** These results establish a coupling between neuronal activity and oxidative metabolism. By making certain assumptions, it is possible to apply these results to fMRI. Specifically, high resolution imaging may be enabled by use of procedures which monitor localized neural activity and this may facilitate columnar level measurements.

■ 4443

## Evidence for distinct ecological constraints of retinal and cortical mechanisms underlying perceptual anisotropies

*SILVA MF (1), LOPES SM (1), GUERREIRO M (1), FARIA PA (1), DUARTE L (2), CASTELO-BRANCO M (1)*
*(1) IBILI/Center for Ophtalmology, University of Coimbra, Coimbra*
*(2) AIBILI/Association for Innovation and Biomedical Research on Light and Image, Coimbra*

**Purpose** Asymmetries of perceptual performance within the visual field have often been attributed to attention and high level visual cortical factors. We have studied human retinal (naso/temporal) and cortical (left/right) perceptual patterns of asymmetry under stimulus conditions that separate the function of magnocellular/parvocellular ganglion cell and rod/cone pathways.

**Methods** Contrast sensitivity performance fields for different test configurations spanning at least 17 visual locations were measured in children (n=80 eyes) and adult (n=88) participants. Magnocellular-biased test stimuli were sinusoidal gratings of 0.25 cpd, vertically oriented and undergoing 25 Hz counterphase flicker. Contrast sensitivity tasks with a significant parvocellular contribution used stimuli at 3.5 cpd and 0 Hz temporal frequency. In this case, both photopic (mean background luminance, 51 cd/m2) and mesopic (0,8 cd/m2) conditions were tested.

**Results** We have observed a nasotemporal pattern of asymmetry with parvo-biased stimulus which is consistent with retinal anatomical anisotropies. An opposite pattern was observed for magno-biased stimuli, demonstrating an additional retinal origin for asymmetric performance fields. Patterns of dorsal-ventral asymmetries were distinct from the classically described cognitive superiority of the inferior visual field. The parvo asymmetry was modulated by a surprising cortical left/right bias.

**Conclusion** We conclude that parvo/magno retinal and cortical mechanisms underlying perceptual anisotropies are driven by different ecological constraints, yielding distinct perceptual magnification factors.

■ 4444

## New methods in examination of achromatopsia

*VARSANYI B (1), SAMU K (2), WENZEL K (2), FARKAS A (1)*
*(1) Semmelweis University, Department of Ophthalmology, Budapest*
*(2) University of Technology and Economics, Budapest*

**Purpose** To examine the retinal structure, and also to analyse the spectral sensitivity of the functioning photoreceptors in patients with achromatopsia.

**Methods** Nine patients with congential achromatopsia were involved in this study; the clinical diagnosis was confirmed by molecular genetic analysis in all cases.Optical coherence tomography (OCT) performs cross-sectional imaging of the retina, providing a semi-histological picture of the anatomical structure via pseudo-colour images.The diagnostic relative brightness measurement (DRBM) is to assess the functioning photoreceptors by comparing the brightness of green-red, blue-red and blue-green fields. Spectral luminosity testing (SLT) measures the maximal sensitive wavelength of a person – it is also characteristic of the functioning photoreceptors.

**Results** The OCT images in patients with achromatopsia showed that the overall structure of their central retina differs from that of normal subjects, and significant reductions in the thickness of the central retina and in the total macular volume were also seen. The results of SLT showed 505-515 nm, characteristic of the rods. In 5 patients DRBM showed proportions characteristic of rods only, but in some cases extreme protanomaly and green monochromacy could be asssumed.

**Conclusion** The new psychophysical methods used in this study could help to distinguish the complete and incomplete forms of achromatopsia. The structural changes seen in the central retina of the patients could provide useful information for the forthcoming gene therapy.

■ 4445

## Rod and Cone Spatial Frequency Sensitivity loss over the first seven years following retinal reattachment

*WEIR R (1, 2), SMITHSON H (2), BIRD A (1, 2), STOCKMAN A (2), SHARPE TS (2)*
*(1) Moorfields Eye Hospital, London*
*(2) Institute of Ophthalmology UCL, London*

**Purpose** To describe and quantify acquired cone and rod loss in spatial frequency sensitivity (SFS) over the first seven years following successful retinal reattachment surgery.

**Methods** We developed a clinical system to measure the scotopic and photopic adapted SFS curve of nine patients who had undergone successful retinal surgical reattachment six months to seven years previously. These new functional assessments of retinal recovery following reattachment were compared with refracted LogMAR visual acuity, OCT-3 images and stereoscopic macula photographs.

**Results** In areas of retinal reattachment, rod SFS curves had steeper high frequency fall-offs and lower overall sensitivity compared with cone. There was a significant difference in the 30 retinal areas studied between affected and unaffected for rod spatial frequency sensitivity but not for cone (Sign test, $p<0.01$); and was apparent at different times following retinal reattachment. Rod SFS loss was unrelated to low levels of lens opacity being consistent in three pseudophakic patients. These findings support the conclusion that the loss in rod sensitivity is due to retinal factors; and were present in eyes that exceeded the minimum legal visual acuity requirement for driving.

**Conclusion** In areas of retinal detachment, rod sensitivity, particularly at higher spatial frequencies, is more profoundly affected after reattachment than cone sensitivity and persists for many years after reattachment. SFS functions are more sensitive markers of long-term retinal damage than corrected LogMAR visual acuity. Reattached retina patients should be advised that navigation and driving by night are likely to be adversely affected despite successful reattachment surgery.

■ 4446

## Comparison of intraocular stray light measurements with conventional cataract grading and contrast sensitivity estimation in elderly European drivers

*MICHAEL R (1), BARRAQUER R I (1), EMESZ M (2), NISCHLER Ch (2), HUFNAGL Ch (2), GRABNER G (2), COPPENS J (3), VAN DEN BERG T J T P (3), COECKELBERGH T (4), WILHELM H (5), VAN RIJN R (6)*
*(1) Institut Universitari Barraquer, Barcelona*
*(2) Landesklinik für Augenheilkunde und Optometrie, Salzburg*
*(3) Netherlands Ophthalmic Research Institute, Amsterdam*
*(4) Universitair Ziekenhuis Antwerpen, Antwerpen*
*(5) Universitäts-Augenklinik Tübingen, Tübingen*
*(6) Vrije Universiteit Medical Center, Amsterdam*

**Purpose** To study the relation between objective stray light measurement with conventional visual function tests.

**Methods** We investigated 2422 random selected elderly drivers in 5 clinics in different EU member states aging between 45 to 85 years and a control group (20 to 30 years). Subjects with prior cataract surgery were excluded (5.7%). Stray light was measured psychophysically applying flicker stray light in a central test field which had to be compensated by another light using a two alternative forced choice strategy. Cataract was graded with the LOCS III without pupillary dilation and contrast sensitivity was measured with a Pelli-Robson chart. Subjects answered the NEI questionnaire VFQ-25 and driving habit questions.

**Results** Stray light values increase from 0.95 (lowest LOCS) to 1.50 (highest LOCS) and decrease from 1.40 (Pelli-Robson <1.20) to 1.00 (Pelli-Robson >1.80). Both relationships are significant and the dependencies have a linear shape. However, drivers reporting night driving difficulties have equal stray light values as drivers without difficulties.

**Conclusion** Objective stray light measurement is well correlated with conventional cataract grading and contrast sensitivity estimation. Individual elevated stray light values are not consistently perceived by the drivers. SUPPORT: EU project: SUB-B27020B-E3-GLARE-2002-S07.18091

## ▪ 4451
## Imaging Diagnosis - New Aspects

*CUNHA-VAZ J (1, 2, 3), SILVA R (1, 2, 3), BERNARDES R (3), BAPTISTA P (3), FERREIRA J (3)*
*(1) Department of Ophthalmology, University Coimbra Hospital, Coimbra*
*(2) IBILI, Faculty of Medicine, University of Coimbra, Coimbra*
*(3) AIBILI, Coimbra*

**Purpose** Advancement in the treatment of Age-Related Macular Degeneration (AMD) depends on the development of new technologies imaging technologies that enable early detection, monitor progression and indicate the best treatment option. We have been able to register Blood-Retinal Barrier (BRB) functional imaging of the human eye to a color fundus photograph of the same eye in a fully automated way. The multimodality involved and fusion of complementary information offers new insights into AMD progression. Presence or absence of invasion of the retinal pigment epithelium outer BRB by choroidal neovascularization progression may determine different therapeutical approaches.

## ▪ 4452
## Update on PDT

*LEYS A*
*Ophthalmology, University Hospitals, Leuven*

**Purpose** In 2005 updated guidelines for the use of verteporfin (Visudyne®, Novartis AG) therapy in the management of patients with choroidal neovascularization (CNV) due to age-related macular degeneration (AMD) and other causes were published in Retina (2005;25:119–134). These were constructed based on data from randomized clinical trials and interpretation by a panel of experts at roundtable meetings in 2003. Recently additional information relevant to clinical care has been published in peer-reviewed literature. Lesion size has emerged as an important predictor of the magnitude of treatment benefit and is a critical factor in the ophthalmologist's decision on whether to treat. Further, verteporfin therapy has also been shown to be beneficial for some patients with minimally classic CNV, leading to indications in all lesion types (predominantly classic, minimally classic and occult with no classic CNV). It is therefore important to consider these factors in the updated guidelines for verteporfin therapy. In addition, early referral when CNV is first diagnosed is paramount in the effective management of patients with CNV due to AMD, as is continual and careful patient follow-up. The treatment of AMD due to CNV is a fast-moving field and it is likely that management guidelines will continue to be revised over the next few years. At present trials are ongoing to study the effect of combination of PDT with triamcinolone and with anti-VEGF agents.

## ▪ 4453
## Update on Lucentis

*BANDELLO FB*
*Department of Ophthalmology, University of Udine, Udine*

**Purpose** Ranibizumab (LucentisTM , RhuFabV2) is a humanized, antigen-binding fragment (Fab) of a second-generation, recombinant mouse monoclonal antibody direct against VEGF. The target of RhuFabV2 is the VEGF where the new vessels grow. It penetrates all retinal layers, binds and inactivates all VEGF isoforms resulting in a reduction of vascular permeability and inhibition of CNV formation. A phase I/II trial evaluated 2 multiple doses of RuFabV2 300 µg or 500 µg every 4 weeks in patients with ARMD complicated by CNV. At 6 months visual acuity was stable or had improved in 93% of patients, had improved by 15 letters or more in 44% of patients and had worsened by 15 letters or more in 7% of patients. Two phase III trials are currently ongoing with RuFabV2: MARINA trial and ANCHOR trial. Patients with minimally Classic/occult lesions were enrolled in the MARINA trial. Patients are being randomized (1:1:1) to receive Ranibizumab (300 µg or 500 µg ) vs a sham injection for 24 months. Patients with predominantly classic choroidal neovascularization were enrolled in the ANCHOR trial. Patients are being randomized 1:1:1 to receive monthly intravitreal Ranibizumab injections of 300 or 500 micrograms or PDT. Patients in Ranibizumab group are eligible to receive additional PDT every 3 months if they show leakage from CNV. Finally there is a phase 3b trial in which patients with subfoveal minimally classic, predominantly classic or occult-only CNV are being randomized 1:1:1 to receive 300 or 500 micrograms of Ranibizumab or a sham injection. Results of MARINA trial are expected in July 2005, and results of ANCHOR trial are expected in December 2005.

## ▪ 4454
## Mechanism of Action of Anecortave Acetate, an Angiostatic Cortisene

*AUGUSTIN AJ*
*Ophthalmology, Karlsruhe*

**Purpose** To review the mechanism of action (MOA) of Anecortave Acetate, an angiostatic cortisene in development for the treatment of age-related macular degeneration (AMD).

**Methods** Anecortave Acetate has been evaluated extensively in fourteen models of neovascularization to determine its MOA and antiangiogenic profile. Chemical modifications to cortisol resulted in the creation of an angiostatic "cortisene" which inhibits blood vessel growth via modulation of several pathways in the 3-step angiogenic cascade: (1) cell damage and activation of the production of growth factors, (2) action of these factors on the vascular endothelial cell (VEC), (3) VEC-growth and new vessel formation. In addition, the molecule is without clinically relevant glucocorticoid receptor activity.

**Results** The molecule acts in various ways to influence the angiogenic cascade. Anecortave Acetate acts in step one via reduction of the expression of proangiogenic factors including VEGF and IGF-1. Ultimately, this action stops VEC activation and the subsequent production of matrix metalloproteinases (MMP's) that degrade the VEC basement membrane and adjacent extracellular matrix. Without this MMP action, proliferation, migration and differentiation of VEC's, new blood vessel growth is inhibited. Anecortave Acetate also works in step three of angiogenesis by blocking the proteolytic cascade. This is performed by up regulating urokinase plasminogen activator (uPA) inhibitor and down regulating uPA and proMMP. In cell culture, Anecortave Acetate blocks both cell proliferation and tube formation.

**Conclusion** Anecortave Acetate blocks angiogenesis by acting at multiple sites of the angiogenic cascade and is shown to prevent neovascularization independent of the inciting stimulus.

## ▪ 4455
## Update on Macugen

*MONES J*
*Instituto de Microcirugia Ocular, Barcelona*

**Purpose** To report 2-year findings from the VISION trials of neovascular age-related macular degeneration (AMD), and highlight benefits of earlier treatment of the disease.

**Methods** Two randomized, double-masked, controlled studies were conducted using broad entry criteria including all angiographic subtypes of AMD and visual acuity of 20/40 – 20/320. Exploratory analyses were also performed using two definitions – arrived at by consensus - of what constituted "early disease": 1) small (<2 disc areas) untreated lesions without fibrosis or atrophy, with baseline VA ≥54 letters, and 2) occult lesions with no classic component, absence of lipid, and VA worse in study eye compared to fellow eye.

**Results** Subjects receiving 2 years of Macugen 0.3 mg had mean loss of 9.4 letters of VA compared to 17.0 letters lost with usual care (P<.05). More subjects who continued 2 years of Macugen 0.3 mg lost < 3 lines of VA (59% compared to 45% on usual care, P<.05). When comparing subjects receiving one year of Macugen to those who received 2 years of Macugen, the second year of therapy afforded additional protection. Sixty seven per cent more occurrences of a 3-line loss of VA were noted in subjects receiving but 1 year of therapy compared to those receiving 2 years. When disease is treated "earlier", as defined above, outcomes are better still. For the 34/294 subjects with "early" disease (definition 1) who received Macugen 0.3 mg and 28/296 subjects on usual care, 76% vs. 50% (respectively) lost < 3 lines at week 54 (P=.03). For the 30/294 subjects with "early" disease (definition 2) on Macugen and 35/296 subjects on usual care, 80% and 57% lost < 3 lines at week 54 (P=.05).

**Conclusion** Compared to both one year of therapy followed by one year of usual care, or 2 years of usual care, a 2 year treatment regimen of Macugen provided sustained therapeutic benefit and better visual outcome as measured at week 102. Early detection and treatment with pegaptanib may result in superior visual outcomes in patients with subfoveal CNV secondary to AMD.

## ▪ 4456
## Corticosteroids in the treatment of AMD

*JONAS JB*
*Dept of Ophthalmology, Faculty of Clinical Medicine Mannheim, Ruprecht-Karls-University of Heidelberg, Heidelberg*

**Purpose** Within the last three years, triamcinolone acetonide has increasingly been applied intravitreally as treatment option for various intraocular neovascular and edematous proliferative disorders.

**Methods** The best response in terms of gain in visual acuity was found in eyes with intraretinal edematous diseases such as diffuse diabetic macular edema, branch retinal vein occlusion, central retinal vein occlusion, and pseudophakic cystoid macular edema. Visual acuity increased and degree of intraocular inflammation decreased in eyes with various types of non-infectious uveitis. Intravitreal triamcinolone may be useful as angiostatic therapy in eyes with iris neovascularisation and proliferative ischemic retinopathies. Possibly, intravitreal triamcinolone may be helpful as adjunct therapy for exudative age-related macular degeneration, possibly in combination with photodynamic therapy. In eyes with chronic, therapy resistant, ocular hypotony, intravitreal triamcinolone can induce an increase in intraocular pressure and may stabilize the eye. The complications of intravitreal triamcinolone therapy include secondary ocular hypertension in about 40% of the eyes injected, cataractogenesis, postoperative infectious and non-infectious endophthalmitis, and pseudo-endophthalmitis. If vision increases and eventually decreases again, the injection can be repeated. Given in a dosage of about 20 mg to non-vitrectomized eyes, the duration of the effect and of the side-effects was 6 to 9 months.

**Conclusion** Intravitreal triamcinolone acetonide may offer a possibility for adjunctive treatment of intraocular edematous and neovascular disorders. One has to take into account the side-effects and the lack of long-term follow-up observations.

## ▪ 4457
## Surgery for AMD

*RICHARD G*
*Department of Ophthalmology, University Medical Center, Hamburg*

**Purpose** Treatment of patients with age-related macular degeneration (AMD) continues to evolve. Different surgical approaches have shown substantial benefit in selected patients with AMD. The methods used are surgical excision of subfoveal CNV, transplantation of retinal pigment epithelium (RPE) and underlying choroid and rotation of the neurosensory retina. There is only limited information based on controlled studies. The data are reviewed with special regard to long-term results and complications.

■ 4461

## Alteration of Keratan sulphate (sulphated poly-n-acetyllactoseamine repeats) in keratoconus cornea: biochemical, histochemical and ultrastructural analysis

*AKHTAR S (1), KERR B (2), HAYES T (2), SALVI, SM (3), HAWKSWORTH NR (3), BRON AJ (4), CATERSON B (2), MEEK KM (1)*
*(1) School of Optometry and Vision Sciences, Cardiff*
*(2) Connective Tissue Biology Laboratories, Departmant of Bioscience, Cardiff*
*(3) Royal Glamorgan Hospital, Cardiff*
*(4) Nuffield Laboratory of Ophthalmology, Oxford*

**Purpose** To investigate the distribution of Keratan sulfate (KS) in normal and keratoconus corneas

**Methods** The distribution keratan sulfate was compared in the stroma of three normal, and one mild keratoconus, and four severe keratoconus corneas. Keratan sulfate was localised using a monoclonal primary antibody (5D4), which recognises linear, pentasulfated hexasaccharide portions of KS. The antigenicity of the antibody was assessed by western blotting and immunogold microscopy.

**Results** Western blotting showed presence of larger number of lower molecular weight products (<25kDa) in keratoconus PG extracts compared to normal PG extracts. Immunogold labelling showed a moderate and uniform distribution of 5D4 in normal cornea. In all keratoconus corneas, labelling was not uniform and was very marked on micro-filament aggregates around keratocytes and within the keratocytes. Quantitative analysis showed that in stroma, the quantity of KS-5D4 was significantly higher (0.001) only in mild keratoconus but not in the severe keratoconus corneas. In the epithelial basement membrane, and Bowman's layer membrane and in keratocytes, the quantity of KS-PG was significantly higher (0.001) in all keratoconus corneas.

**Conclusion** The finding of an altered expression of KS in our keratoconus corneas, in particular the strong expression of KS in keratocytes, is in keeping with reports of altered proteoglycan metabolism in keratoconus. Keratan sulfate plays an important role in stromal collagen fibril assembly. A dysregulation of KS synthesis could explain the changes in collagen fibril spacing and diameter, which we have reported elsewhere.

■ 4462

## Presence of corneal neovascularization is less important factor for corneal graft survival than allo-sensitization with previous allograft

*GUO A, KUFFOVA L, FORRESTER JV*
*Department of Ophthalmology, University of Aberdeen, Aberdeen*

**Purpose** Corneal allografts experience immune privilege. However, the privilege is only relative, where some grafts, especially the ones with previously rejected corneal allograft are more prone to rejection and failure than first allografts. Two factors may contribute to these grafts failures: previous trauma and systemic sensitization. In this study we compared two "high risk" models (1) recipients with suture-induced corneal neovascularization (2) recipients with previously rejected corneal allograft

**Methods** BALB/c (H2d) mice were used as recipients and donors. C57BL/6 (H2d) and CBA/CA (H2k) corneas were transplanted to prevascularized BALB/c (H2d) recipient or to naïve BALB/c mice. 8 weeks later the same recipients were re-grafted with corneal allograft, syngeneic graft or third party graft. The strain combinations were incompatible across both MHC and non-MHC antigens.

**Results** A second allograft from the original donor performed in recipients with a previously rejected corneal graft, was rejected with greater speed. In contrast, speed of rejection was not significantly accelerated when allografts were placed in suture-induced neovascularised recipient beds compared to the survival of the primary allografts. The accelerated rejection of second grafts was alloantigen specific since a second graft from a third party donor did not show significant difference in speed of rejection compared to first allograft. Syngeneic grafts remained accepted and clear under all circumstances.

**Conclusion** Trauma-induced neovascularisation of the cornea is a less important risk factor for corneal graft rejection than prior allo-sensitization with corneal allograft.

■ 4463

## Comparison of Real Time Intraocular Pressure During LASIK Using Two Different Keratomes in Pig Eyes; Moria 2 vs. INTRALASE

*HERNANDEZ VERDEJO JL (1, 2), BOLIVAR G (3), ROMAN JM (1), TORRES J (1), TELIS MA (1, 4)*
*(1) VISSUM Hospital Oftalmológico Madrid, Madrid*
*(2) Universidad Complutense de Madrid, Madrid*
*(3) Hospital Universitario Príncipe de Asturias, Madrid*
*(4) Universidad de Alcalá, Madrid*

**Purpose** To compare real time intraocular pressure (IOP) during LASIK measured by a baro transducer using two different types of microkeratomes, in pig eyes.

**Methods** This is an interventional, prospective study. Two different devices were used.; a Moria 2 (group 1) and a femtosecond device (Intralase, group 2) devices were employed in each group to create a lamellar corneal flap in freshly enucleated pig eyes. The intraocular pressure changes induced by the suction ring on each eye were recorded using a reusable blood pressure transducer, connected with the anterior chamber by direct cannulation. IOP was monitored while the flap was created either in both groups.

**Results** 7 pig eyes were studied in each group.IOP increased during the suction, reaching a mean of 122,53mmHg±30,40, and during the cut, reaching 160,52 mmHg ±22,73 in group 1. On the other hand, in group 2, IOP reached 89,24mmHg±24,57 during the suction, and 119,33 mmHg±17,01 during the intraestromal laser application. Both levels of IOP were statistically different between the two groups (p=0.01).

**Conclusion** Real time IOP can be measured during keratomileusis using a transducer connected with the anterior chamber. Our results show a significant increase in IOP during the procedure in both groups, although Intralase seems to increase the IOP to a lesser extent than a conventional, mechanical microkeratome.

■ 4464 / 241

## Immunomodulatory Role of VIP in a Murine Model of P. aeruginosa-Induced Keratitis

*SZLITER EA, LIGHVANI S, HAZLETT LD*
*Anatomy & Cell Biology, Detroit*

**Purpose** In P. aeruginosa ocular infection, dominant Th1-responsive mouse strains are susceptible (cornea perforates), while Th2-responsive mouse strains are resistant (cornea heals). Neuropeptides are endogenous neuroendocrine factors recently correlated with neuroimmune interaction and function, but little is known of their role in mediating/regulating ocular infectious disease. Previous studies have shown an increased level of vasoactive intestinal peptide (VIP) expression, a neuropeptide associated with potent anti-inflammatory activities, in BALB/c (Th2-responder) vs. C57BL/6 (B6) (Th1-responder) mice. Therefore, this study further examined the role of VIP in modulating the host immune response to P. aeruginosa-induced keratitis.

**Methods** B6 mice were injected i.p. with recombinant (r) VIP daily from -1 through 7 days p.i. Control mice were similarly injected with PBS. Clinical scores, slit-lamp, bacterial plate counts, myeloperoxidase to quantitate PMN, real-time RT-PCR and ELISA were used to assess the effects of rVIP treatment in modulating disease pathogenesis.

**Results** Injection of B6 mice with rVIP prevented corneal perforation, normally seen in the susceptible animal by 5-7 days p.i. Real-time RT-PCR analysis showed significantly decreased levels for both IFN-g and IL-1b mRNA at 7 days p.i. in rVIP corneas. Bacterial load was comparable for rVIP vs. control mice, while fewer PMN were present with time in rVIP-treated mice.

**Conclusion** These data provide evidence that VIP is a regulatory molecule of ocular inflammation. rVIP treatment counterbalanced the production of pro-inflammatory cytokines (IFN-g and IL-1b) and down-regulated PMN infiltration sufficiently to ameliorate the host-induced component of bacterial-induced inflammatory disease.

## ▪ 4465 / 242
## Corneal toxicity study of Perfluorohexyloctane in rabbit eyes

*DIAFAS SG (1), LAKE S (1), KARAMPATAKIS VE (2), KARDASOPOULOS AV (1), STANGOS NT (1), MANTHOS AK (3)*
*(1) Ophthalmology, Aristotle University of Thessaloniki, Thessaloniki*
*(2) Laboratory of Experimental Ophthalmology, Aristotle University of Thessaloniki, Thessaloniki*
*(3) Laboratory of Histology-Embryology, Aristotle University of Thessaloniki, Thessaloniki*

**Purpose** To evaluate the effects of perfluorohexyloctane (F6H8), a semi-fluorinated fluorocarbon of low specific gravity, on the rabbit cornea.

**Methods** 6 pigmented rabbits underwent injection of 0.1 ml of F6H8 in the anterior chamber of the right eye and BSS in the left eye. The rabbits were sacrificed after 24 days, the eyes were then enucleated and 1 specimen from the upper and 3 from the lower cornea of each eye were taken and processed for transmission electron microscopy (TEM). The specimens of the inferior cornea in contact with the F6H8 were then compared with the specimens of the superior cornea.

**Results** Postoperatively the inflammation in eyes receiving F6H8 was more severe than in the controls. In the anterior chamber, the formation of small bubbles, "fish egging" phenomenon, was observed. A ridge was observed in 2 eyes, as a landmark limiting the area of contact with the F6H8, with conjunctival congestion and cornea cloudiness, without corneal neovascularization.On TEM all the control eyes (BSS) were free of pathological changes. Morphological lesions were found in the area in contact with the F6H8 in the inferior cornea. Vacuoles of F6H8 were present within the endothelium but not in the corneal stroma. Widening of the endothelial microvilli was present and also flattening of the endothelium cells and their nuclei. Formation of a multi layer retrocorneal fibrous membrane was observed in conjunction to the endothelial cells in 2 eyes.

**Conclusion** Our data indicate that F6H8 induces corneal toxicity in rabbits when present in eyes for 24 days. We therefore recommend caution in patients without barrier to prevent the fluid migrating from the vitreous cavity into the anterior chamber.

## ▪ 4466 / 243
## Corneal Crystallin Expression in Human Repair Phenotype Keratocytes

*McDERMOTT AM, PEI Y, SHERRY DM*
*College of Optometry, Houston*

**Purpose** To compare expression of the putative corneal crystallins aldehyde dehydrogenase (ALDH) and transketolase (TKT) in human corneal fibroblasts, myofibroblasts and penetrating keratoplasty (PKP) specimens.

**Methods** Isolated human keratocytes were cultured with serum or serum and TGF-beta to obtain corneal fibroblasts and myofibroblasts respectively. RT-PCR, immunoblotting and labeling were used to detect expression of ALDH isoforms and TKT. ALDH3A1 activity was quantitated by a colorimetric assay. PKP tissue sections were immunolabeled for ALDH3A1, Thy-1 (a repair phenotype marker) and alpha-smooth muscle actin (alpha-SMA, a myofibroblast marker).

**Results** Keratocytes expressed ALDH 1 and 3A1 mRNA (n=3), and 1 of 3 samples expressed ALDH 2 and TKT. Corneal fibroblasts expressed ALDH 1 and 3A1 mRNA (n=3) and there was a 26% decrease in ALDH3A1 mRNA expression in fibroblasts compared to keratocytes (n=2). ALDH3A1 protein was readily detectable in keratocytes but was not present in corneal fibroblasts or myofibroblasts (n=3). ALDH3A1 enzyme activity was markedly lower in fibroblasts than keratocytes (14 +/- 12 vs 135.6+/-73 U/mg protein, n=3). Repair phenotype keratocytes (Thy-1 or alpha-SMA positive) were present in 2 of 6 PKP specimens. These cells did not stain for ALDH3A1 whereas all normal keratocytes (Thy-1 or alpha-SMA negative) were ALDH3A1 positive.

**Conclusion** These results confirm in human cornea, the results of previous animal studies showing decreased ALDH3A1 expression in repair phenotype keratocytes. The significance of the decrease remains to be determined but may contribute to enhanced reflectivity of the repair phenotypes and hence stromal haze.

## ▪ 4467 / 244
## Numerical simulation of corneal transport processes

*TIGHE B, LI LY*
*School of Engineering and Applied Science, Birmingham*

**Purpose** Modeling of corneal swelling and its environmental dependence is critical to an understanding of corneal function, particularly when important physiological parameters are refractory to experimental investigation. For the cornea, endothelial membrane transport parameters are difficult to access directly, thus development of an effective stromal transport model will enable isolation of endothelial membrane transport properties.

**Methods** Transport equations for each ionic species and ionic solution within the corneal stroma are derived based on transport processes developed for electrolytic solutions, whereas the transport across epithelial and endothelial membranes is modeled using phenomenological equations derived from thermodynamics of irreversible processes. Time-dependent effects are then derived.

**Results** For given initial ionic concentrations and stromal hydration levels together with boundary conditions (ionic concentrations and hydrostatic pressures in tears and aqueous humour) partial differential equations are solved using finite element methods, to obtain the hydration distribution, and thus the thickness variation. . The greatest change in thickness is found for the combination of sodium and bicarbonate ions whilst least change is found with the combination of potassium and chloride.

**Conclusion** A computational model of corneal hydration has been proposed for simulating the response of corneal thickness to perfusion with hypotonic or hypertonic solutions.This has been used to demonstrate the influence of flow across the epithelium and endothelium on the stromal thickness response and the role of the ionic active pumps in epithelial and endothelial layers.

## ▪ 4468 / 245
## Cellular activity in human regrafts 1 – 30 years primary surgery

*GAN L (1), FAGERHOLM PPP (1), STENEVI U (2)*
*(1) Ophthalmology, Linköping*
*(2) Ophthalmolofy, Göteborg*

**Purpose** A previous study by the group have revealed that the cells in 60 studied human corneal grafts are to a large extent replaced also in the stroma and in the endothelium. The time to reoperation was between 1 and 30 years. The interest of this study was to see cellular activities in these corneas at the time of retransplantation.

**Methods** We used immunohistochemichal markers for proliferative cell nuclear antigen (PCNA), Caspase 3, S 100 A4, PINCH and VEGFr-3 to study the expression in formaldehyde fixed, paraffin embedded corneal buttons retrieveded at reoperation1-30 years after the primary transplantation. Eye bank corneas and 3 corneal buttons retrieved at primary corneal grafts surved as reference material.

**Results** PCNA was expressed in all cell layers in the majority of the 19 specimens. 46 cells/ section were positive in the epithelium and 33 cells/ section in the stroma on average. In the endothelium, 11 % of the endothelial cells expressed PCNA. These figures were slightly higher in the 5 reference corneas. Caspas 3 expression was found in half of the specimen in all cell layers but very few in numbers. There was a discrepancy in the expression of S 100 A4 and PINCH in that S100 A4 expressed extensively and PINCH rather restricted both in all cell types.VEGFR-3 was expressed in all the rejected grafts as well as other grafts which regrafted by others reasons. The cell types of VEGFR-3 positivity found are case related.

**Conclusion** The cell proliferative activity can be seen in regrafts in all cell types. The apoptotic activity exists but is scarce. Markers for mobility, S 100A4 and PINCH, expresses differently in different cases. VEGFr-3 is involved in human regrafts. The role of VEGFr-3 in corneal immune-rejection need further studies.

## ▪ 4469 / 246
## Evaluation of Megacell MEM as a corneal storage medium

*SMITH VA*
*Ophthalmology Unit, Bristol*

**Purpose** Bristol CTS Eye Bank corneas are currently held in MEM containing 2% FCS. Endothelial cell loss could be reduced by increasing the FCS concentration to 10%. By repute, Megacell MEM reduces the FCS dependence of cultured cells. The aim of this project was therefore to evaluate Megacell MEM for storing corneas.

**Methods** Rates of keratocyte proliferation and epithelial cell outgrowth from limbal explants in MEM or Megacell MEM were determined using Alamar blue. Paired corneas were maintained in either MEM or Megacell MEM containing 2% FCS and their endothelial cell densities were estimated over a period of 6 weeks.

**Results** The proliferation rates and viability of corneal keratocytes and epithelial cells were similar in Megacell MEM containing 2% FCS and MEM with 10% FCS. For corneas maintained in Megacell medium the initial rates of endothelial cell loss (weeks 0-3) were similar to the rates of cell loss between weeks 3-6. For corneas maintained in MEM the rates of endothelial cell loss were dependent upon pre-experimental storage time. For those stored for under 10 days the initial rates of cell loss were significantly less than the rates between weeks 3-6 and similar to those of corneas maintained in Megacell medium. For those stored for 10-16 days the initial rates of endothelial cell loss and the rates between weeks 3-6 were similar but significantly higher than those of corneas maintained in Megacell MEM. Overall the morphological appearance of the corneas in Megacell MEM was markedly better than those in MEM.

**Conclusion** Megacell MEM reduced the FCS dependence of corneal keratocytes and epithelial cells in culture and prolonged the viability of the endothelia of corneas destined for transplantation. It is therefore a better storage medium for these corneas than MEM.

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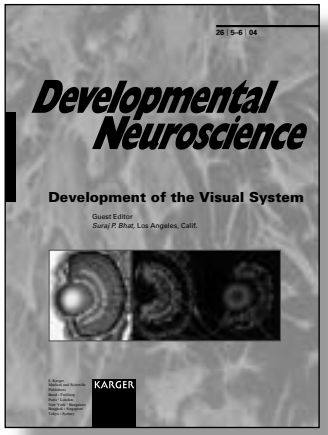


# Development of the Visual System

**Editor**
**Suraj P. Bhat**

Neurology; Neurobiology; Ophthalmology; Cell Biology, Embryology, Molecular Biology

**Contents**



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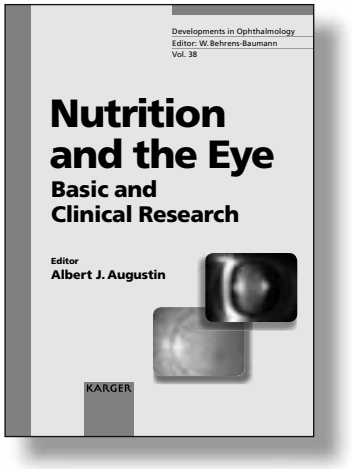


# Nutrition and the Eye

**Editor**
**Albert J. Augustin**

Ophthalmology; Biochemistry; Nutrition; Pharmacology; Natural Sciences for Physicians

**Contents**



This volume contains a basic research section focusing on the major compounds of nutrition and food supplements as well as a clinical research section providing up-to-date information on the results of recent clinical studies.
The first part gives an insight into the mechanisms of substances relevant to antioxidants and food supplements in relation to eye diseases. The consequences and relevance of selenium, one of the most important trace elements, are considered in a separate section. Further, vitamins E and C as well as lutein and zeaxanthin, the physiological macular pigment, are discussed.
The second part focuses on both anterior and posterior segment diseases which might be influenced by food supplementation and/or antioxidants. In addition, this section explains the oxidative pathomechanisms of the most important disease processes.
Written for clinicians as well as basic vision scientists, this volume is an essential contribution to the research activities, especially in eye diseases leading to blindness such as diabetic retinopathy and age-related macular degeneration.

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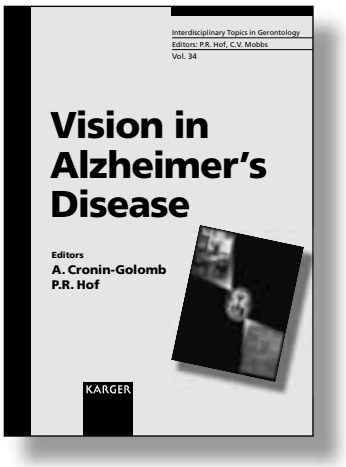


# Vision in Alzheimer's Disease

**Editors**
**Alice Cronin-Golomb**
**Patrick R. Hof**

Gerontology; Neurology; Psychology; Geriatrics, Ophthalmology, Psychiatry, Neuropathology, Neurobiology

Visual dysfunction is prevalent in Alzheimer's disease and in related disorders such as posterior cortical atrophy and Down syndrome. The neuropathology of these disorders affects brain areas that process low-level vision as well as higher-order cognition and attention.

This volume spans the range of topics on vision, from structure (retinal and cortical) to function (cortical activation) to behavior (perception, cognition, attention, hallucinations, and everyday activities). The chapters together indicate that lower-level visual deficits can contribute to, or masquerade as, higher-order cognitive impairments. As important, they suggest that vision-based interventions may improve patients' lives. An emerging theme is that the study of variations in visual-system pathology, behavior, and genetic risk will likely provide insights into typical Alzheimer's disease as well as related conditions. The visual disorders of Alzheimer's original case and its cousins of the 21st century have much to teach us about the changing visual system in aging and age-related neurodegenerative disease.

This book is essential reading for neurologists, neuropsychologists, ophthalmologists and optometrists, geriatricians and gerontologists, psychiatrists, occupational therapists and other health professionals who provide diagnosis and clinical care to individuals with Alzheimer's disease and related disorders.

**Contents**



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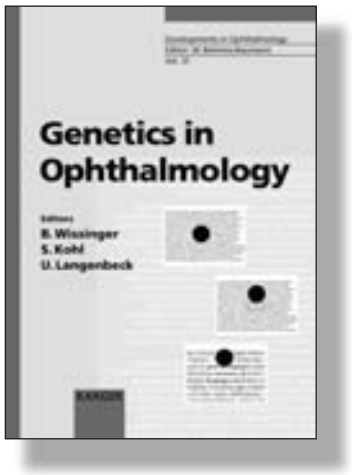
A comprehensive survey of the state of the art in molecular analysis, epidemiology and the management of hereditary ocular disorders




# Genetics in Ophthalmology

**Editors**
**B. Wissinger**
**S. Kohl**
**U. Langenbeck**

Ophthalmology; Genetics; Epidemiology, Human Genetics, Molecular Biology, Neurobiology

The objective of this publication is to enhance mutual understanding and communication between ophthalmologists, molecular geneticists, genetic counselors and biomedical researchers.

In the introductory chapter, current genetic paradigms and experimental genetic approaches relevant to the nature of hereditary disorders are discussed. The following contribution on the epidemiology of hereditary ocular disorders provides an excellent reference to geneticists as well as clinicians. Myopia is presented as an example of a complex clinical phenotype where genes and environment interact. Further molecular ophthalmogenetic topics, such as corneal dystrophies, cataract, glaucoma, opticus neuropathy, non-syndromic and syndromic pigmentary retinopathies, defects of vitamin A metabolism and macular dystrophies including age-related macular degeneration, are investigated in depth. The volume concludes with a survey of color vision deficiencies, a discussion of animal models and gene therapy, and a useful description of technical devices supporting patients who are losing sight.

**Contents**



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