



ABSTRACTS



2006 | October 4 - 7, 2006
Vilamoura, Portugal

www.ever.be



2006 | October 4 - 7, 2006
Vilamoura, Portugal

www.ever.be

KEYNOTE LECTURES

• Erik DE CLERCQ	6
• Nicholas GALLOWAY	7
• Anthony MOORE	8
• Paul SIEVING	9
• Jialiang ZHAO	10
• Narcing RAO	11
• Charlotte REME	12

POSTERS

• Posters 201-258, exhibited on Thursday	14 - 28
• Posters 301-386, exhibited on Friday	29 - 50
• Posters 401-486, exhibited on Saturday	51 - 72

COURSES

• Course 1	74
• Course 2	75 - 76
• Course 3	77
• Course 4	78
• Course 5	79

ORAL PRESENTATIONS

• Sessions on Thursday	82 - 125
• Sessions on Friday	126 - 168
• Sessions on Saturday	169 - 204

All Authors Index

205

TABLE OF CONTENTS



2006 | October 4 - 7, 2006
Vilamoura, Portugal

www.ever.be

KEYNOTE LECTURES



■ 1001

Antivirals for viral eye infections

*Erik DE CLERCQ**Rega Institute for Medical Research, KU Leuven, Leuven*

The current armamentarium for the chemotherapy of viral infections consists of approximately 40 licensed antiviral drugs. For the treatment of human immunodeficiency virus (HIV) infections, 21 compounds have been formally approved: (i) the nucleoside reverse transcriptase inhibitors (NRTIs) zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir and emtricitabine; (ii) the nucleotide reverse transcriptase inhibitor (NtRTI) tenofovir disoproxil fumarate; (iii) the non-nucleoside reverse transcriptase inhibitors (NNRTIs) nevirapine, delavirdine and efavirenz; (iv) the protease inhibitors saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir (combined with ritonavir at a 4/1 ratio), atazanavir, fosamprenavir and tipranavir, and the viral entry inhibitor enfuvirtide. For the treatment of chronic hepatitis B virus (HBV) infections, lamivudine, adefovir dipivoxil and entecavir have been approved. Among the anti-herpesvirus agents, acyclovir, valaciclovir, penciclovir, famciclovir, idoxuridine, trifluridine as well as brivudin have been approved for the treatment of herpes simplex virus (HSV) and/or varicella-zoster virus (VZV) infections; and ganciclovir, valganciclovir, foscarnet, cidofovir and fomivirsen (the latter upon intravitreal injection) have been approved for the treatment of cytomegalovirus (CMV) infections in immunosuppressed patients (i.e. AIDS patients with CMV retinitis). Following amantadine and rimantadine, the neuraminidase inhibitors zanamivir and oseltamivir have recently become available for the therapy (and prophylaxis) of influenza virus infections. Ribavirin has been used (topically, as aerosol) in the treatment of respiratory syncytial virus (RSV) infections, and the combination of (oral) ribavirin with parenteral (pegylated) interferon-alpha has received increased acceptance for the treatment of hepatitis C virus (HCV) infections.



■ 1002

The Evolution of Electrical Responses from the Eye

Nicholas GALLOWAY

University Hospital Queen's Medical Center, Nottingham



■ 2001

Cone and cone rod dystrophies, genotype, phenotype and disease mechanisms

Anthony MOORE

Institute of Ophthalmology, University College, London

The cone and cone-rod dystrophies are a heterogeneous group of disorders, both in terms of clinical features and underlying molecular genetic basis. These inherited retinal disorders are characterised by visual loss, abnormalities of colour vision, and a variable degree of nystagmus and photophobia. There is absent or severely impaired cone function on electroretinography (ERG). The cone dystrophies can be usefully divided into stationary (cone dysfunction syndromes) and progressive disorders. The cone dysfunction syndromes often present shortly after birth or in infancy whereas progressive cone dystrophies usually present in childhood or early adult life; most of the latter group later develop additional rod system abnormalities that lead to night-blindness later in the disease process. Many different phenotypes can be identified using electrophysiological and psychophysical testing. In recent years there have been considerable advances in our understanding of the molecular genetic basis of this group of disorders. Genes encoding a diverse group of proteins including components of the photo transduction cascade, transcription factors, ion channels and synaptic proteins have been implicated. This talk will review recent advances in understanding of the cone dysfunction syndromes and cone dystrophies.



■ 2002

The Interplay of Clinical and Basic Knowledge in X-linked Retinoschisis

*Paul SIEVING**National Eye Institute, National Institutes of Health, Bethesda*

Juvenile X-linked retinoschisis was first described by Dr. Josef Haas in 1898 in two brothers. The retinoschisis gene was cloned a century later, in 1997, and was found to encode a small 224 amino-acid protein containing a discoidin domain that mediates biological "adhesiveness." During the decade since, up to 2006, biological concepts about this genetic retinal dystrophy have changed fundamentally, and the clinical understandings of disease pathology have also undergone concurrent evolution. Cell biological studies since gene cloning have shown that photoreceptors express retinoschisin protein abundantly, which was surprising, as classically the condition was known to cause retinal delamination through the ganglion cell layer. Further studies demonstrated that all retinal neurons express retinoschisin, including ganglion cells, amacrine cells, bipolar cells, and photoreceptors. The XLRS gene knockout mouse, created in the laboratory, deviated remarkably from the classical understanding of human XLRS disease in showing cellular and plexiform involvement of all retinal layers. When ocular coherence tomography was developed during the 1990s, this showed that human XLRS pathology mirrored the laboratory mouse and involved all retinal layers. AAV delivery of the XLRS gene into the XLRS knockout mouse rescued the electroretinogram signal even in the adult animals, lending hope that human gene delivery may prove therapeutic in the future. Although X-linked retinoschisis is only a single example of one Mendelian genetic hereditary trait, similar evolution of basic and clinical disease understanding is occurring across the ophthalmic landscape and is pointing us toward fundamental new ways to treat eye diseases.



■ 3001

Advance of Ophthalmology in China and the Way Forward to International Ophthalmology

Jialiang ZHAO

Dept of Ophthalmology, Peking Union Medical College Hospital, Beijing

The origin of Chinese Ophthalmology can be traced back to traditional Medicine more recently supplemented with Western Ophthalmology. Since the foundation of new China in 1949 there has been rapid development of modern ophthalmology in China. There are currently 3,700 Ophthalmic organizations and 22,000 Ophthalmologists in mainland China. The Chinese Ophthalmological Society (COS) plays a very important role in the development of Ophthalmology. The scale of the COS Congress has increased to about 4,500 participants. There has been a National examination for residents in Ophthalmology since 2001. Modern techniques and therapies are now widely applied in China. Ophthalmic microsurgery, including modern cataract extraction and IOL implantation, vitrectomy and glaucoma surgery have become commonplace. The percentage of IOL implantation is more than 80%. Laser therapy for glaucoma, retinal diseases and other diseases, and excimer laser refractive surgery are widely applied. In the last 20 years, a group of basic researchers in Ophthalmology and Visual Science has been organised in China, some of whom have achieved high academic status. There is a massive program for prevention and epidemiological studies of eye diseases underway. Infectious eye diseases, such as trachoma, and Vitamin A deficiency have been controlled. A network for surgical treatment of cataract blindness has been formed. The Chinese Ophthalmological Society fully recognises that these achievements would not have happened without the support of the wider International Ophthalmological Community. We also recognise that a combined approach will benefit the development of Ophthalmology in China. To this aim, the COS are making great efforts and look forward to working together with Ophthalmologists all over the world.



■ 3002

Photoreceptor Mitochondrial Oxidative Stress in Experimental Uveitis

Narciso RAO

Doheny Eye Institute, Los Angeles

Purpose Uveitis, a leading cause of blindness resulting from the degeneration of photoreceptors has been studied extensively in animal model, Experimental Autoimmune Uveitis (EAU). Purpose of the study is to test a hypothesis that prior to macrophage and other phagocytic cell infiltration in the retina in EAU, photoreceptor degeneration begins with selective cytochrome c nitration as a consequence of nitric oxide species generation in the photoreceptor mitochondria.

Methods During early phase of EAU, prior to the leukocytic infiltration in the retina, enucleated eyes were processed to detect generation of nitric oxide derived reactive species(ONOO⁻) by immunohistochemistry, immunoblotting and mass spectrometry; and generation of cytokines in the retina by Affymetrix followed by real time PCR. The reactive species were localized by immunohistochemistry utilizing confocal microscopy. The photoreceptor damage was detected by TUNEL method. Chemotactic activity of the nitrated proteins and photoreceptor hydroperoxides was determined.

Results During the early EAU there was upregulation of T cell derived cytokines, particularly those involved in induction of nitric oxide synthetase (NOS). There was selective upregulation of NOS in the photoreceptor mitochondria and nitration of cytochrome c. Although there was absence of apoptotic photoreceptors during the early phase, apoptotic photoreceptors were present during amplification phase of the uveitis. The nitrated proteins and lipid hydroperoxides were chemotactic to macrophages and microglia.

Conclusions The results suggest that photoreceptor mitochondrial oxidative stress could be initial central event in photoreceptor degeneration leading to mobilization of microglia and recruitment of the inflammatory phagocytes.



■ 4001

Light damage and retinal degenerations: a model that sometimes tells the truth

Charlotte REME

Dept. Ophthalmology, University Hospital, Zurich

Purpose Light damage occurs in the human retina and is abundantly investigated in animal models. Chronic low level light damage is suspected as contributory in age related macular degeneration (AMD). Light exposure has been shown to enhance retinal degeneration in animal models of human retinitis pigmentosa (RP). Here, mechanisms of light damage will be shown and discussed in view of a model system as well as in light of retinal degenerations.

Methods We use short term exposures to bright white light in freely moving rodents or acute, short term exposure to monochromatic light of different wavelengths in anesthetized rodents. We analyse morphology, function, rhodopsin regeneration kinetics and molecular biological parameters. We apply pharmacological tools to obtain neuroprotection with the aim of reducing or even preventing visual cell- and pigment epithelial cell loss.

Results In retinal degenerations as well as in light damage, visual cells die by apoptosis. The major chromophore is rhodopsin, since rodents which cannot synthesize 11 - cis retinal (Rpe65-/- mice) are not damaged. Modifying factors for white light - lesions include fatty acid composition of rod outer segments, rhodopsin regeneration rate and the pro - apoptotic transcription factor AP1 (activator protein 1). Blue light - lesions are independent of AP1, phototransduction and shut off but depend on body temperature and wavelength.

Conclusions Acute light damage in humans is known to clinicians as solar retinopathy. Epidemiological studies concerned with life long light exposure as risk factor for AMD need to be revised in view of recent findings of genetic variants in the complement system (Y402H) and C-reactive protein, because those variants which confer highly significant risk have not been previously controlled. Studies in animal models should increase clinical awareness of potential risk for RP patients bearing certain rhodopsin mutations (P23H, T4R, T17M) to suffer from lesions by ophthalmological instruments or other light sources.



2006 | October 4 - 7, 2006
Vilamoura, Portugal

www.ever.be

POSTERS

■ 201 / 2336

Ectasia After LASIK; Case Report

ABAHUSSIN M (1), VELARDE J (2), MEEK KM (1), COTERO J (2)

(1) School of Optometry and Vision Sciences, Cardiff University, Cardiff

(2) The Cantabrian Institute of Ophthalmology, Spain

Purpose To study the ultra-structural features of a post-LASIK ectatic cornea from a low myopic patient.

Methods X-ray diffraction (XRD) was used to study collagen fibril orientation within the stroma of a normal cornea and a grafted 7mm post-LASIK ectatic corneal button with a residual stromal bed thickness of about 350 microns. Scanning electron microscopy (SEM) and light microscopy (LM) was used to further characterise the ultra-structural features of the ectatic cornea.

Results The normal preferred orthogonal orientation of collagen fibrils in the superior-inferior and nasal-temporal directions is unaltered in post-LASIK ectatic tissue. SEM revealed a thinning and unknown defect at the inferior part of the cornea. Moreover, during SEM preparation, the LASIK-flap separated from the remaining cornea thus indicating that after four years, the flap still does not contribute to biomechanical integrity of the cornea.

Conclusion Post-LASIK ectasia is not associated with any change in collagen orientation. Tissue thinning in post-LASIK ectasia occurs only within the intact stromal bed; flap thickness remains unaltered. The detachment of the flap from the stromal bed during SEM processing, which utilises chemicals known to cause cellular disruption, suggests that cellular rather than matrix components are involved in flap adhesion.

■ 202 / 2337

Metabolic and Molecular Signalling Pathways of Retinoids on Human Trabecular meshwork

SAPIN V (1, 2), BLANCHON L (1), MARCEAU G (1, 2), NEZZAR H (3, 1),

DASTUGUE B (2), RIGAL D (3, 1), YUÉ B (4), CHIAMARETTA F (3, 1)

(1) ARDEMO, Clermont Ferrand

(2) INSERM U.384, Clermont Ferrand

(3) Department of Ophthalmology, Clermont Ferrand

(4) University of Illinois, Chicago

Purpose The integrity of the human ocular surface has an absolute requirement of vitamin A and its active derivatives, the retinoic acids. These retinoids act through the activation of nuclear receptors: RARs and RXRs. These receptors have been studied in rabbit, mouse and human cornea, but tissue and cellular expression of the 3 RARs and 3 RXRs had to be established at the human trabecular meshwork. The first objective of this work was to define their expression patterns in term of genes and proteins. The second objective was to demonstrate the presence of different enzymes transforming vitamin A to retinoic acid and the activity of this retinoids pathway in the ocular surface.

Methods Total mRNA was extracted from total human trabeculum meshwork, primary cell cultures of human trabecular meshwork established from donor corneas, and submitted to RT-PCR experiments. Immunological staining experiments were performed to better define the RARs and RXRs protein localization. The expression patterns of retinal dehydrogenase (RALDHs) and alcohol dehydrogenase (ALDHs) was also determined in human trabecular meshwork cells. The metabolic pathway is tested by targeted disruption of different enzyme steps transforming retinol to retinoids.

Results RAR alpha, beta, gamma, RXR alpha, ALDH4 and RALDH2 are expressed in trabecular meshwork. Trabecular cell meshworks are able to convert retinol into retinoic acid.

Conclusion For the first time, we established an exhaustive description of RARs and RXRs, ALDHs and RALDHs expression patterns, in the human trabecular meshwork. Our results demonstrated in the human trabecular meshwork, the presence of all the actors of retinoic acid signalling pathway and their activation.

■ 203

Influence of Toll-like receptor agonists on IL-6 expression in human retinal pigment epithelial cells (ARPE-19)

PAIMELA T (1), RYHÄNEN T (1), SALMINEN A (2), UUSITALO H (1), KAARNIRANTA K (1)

(1) Depth of Ophthalmology, Kuopio

(2) Depth of Neurology and Neurosciences, Kuopio

Purpose To study influence of Toll-like receptor agonists on IL-6 expression in human retinal pigment epithelial cells (ARPE-19).

Methods Inflammatory response of ARPE-19 cells to various Toll-like receptor agonists such as Pam, Zymosan, Flagellin, SLTA and Lipopolysaccharide (LPS) exposures were recorded by the secretion of cytokine IL-6 as analyzed by ELISA. The responses of 17beta-estradiol, ICI 182,780 (estrogen receptor inhibitor) and Helenalin (NF-kappaB inhibitor) were compared to the IL-6 expression levels.

Results Toll-like 2 and -4 receptor agonists Pam and LPS, respectively, caused strong IL-6 response in ARPE-19 cells. 17beta-estradiol clearly attenuated the inflammatory response in both preconditioning and simultaneous treatments with the receptor agonists. However, anti-inflammatory role of 17beta-estradiol was not mediated through estrogen receptors. Interestingly, NF-kappaB inhibitor clearly reduced Pam- and LPS-induced IL-6 expression levels. Other used Toll-like receptor agonists did not affect on IL-6 protein levels.

Conclusion Our results show that 17beta-estradiol has anti-inflammatory effects against Toll-like 2 and -4 receptor stimulation. However, it seems that this response is not estrogen receptor-mediated. In addition, this study reveals that NF-kappaB transcription factor regulates IL-6 expression in ARPE-19 cells.

■ 204

Geldanamycin modulates ubiquitin-proteasome pathway mediated protein aggregation in human ARPE-19 cells

RYHÄNEN T (1), KAARNIRANTA K (1), KUUSISTO E (2), MIETTINEN R (2), SALMINEN A (2), UUSITALO H (1)

(1) Depth of Ophthalmology, Kuopio

(2) Depth of Neurology and Neurosciences, Kuopio

Purpose Retinal pigment epithelial cells (RPE) maintain survival of photoreceptors. One of the essential functions of RPE cells is to break down membranous discs shed from photoreceptor outer segment. It has estimated that lysosomal proteolysis takes care of 20%, while proteasomes degrade 80% of cellular proteins. Proteins that are undergoing to proteasomes are tagged with ubiquitin. In response to various stresses, cells increase the expression of heat shock proteins (Hsps). They function as molecular chaperones, in order to prevent the accumulation of cellular cytotoxic protein aggregates. Role of Hsp90, Hsp70, ubiquitine and proteasome inhibition were evaluated in cellular aggregation in human RPE cells (ARPE-19).

Methods Cellular localization of Hsp90, Hsp70 and ubiquitin were studied by immunofluorescence and phase contrast microscopy. Transmission electron microscopy was used to detect cellular organelles in ARPE-19 cells. Cell viability was analyzed by MTT-assay.

Results Electron microscopy showed a robust accumulation of juxtanuclear protein aggregates in response to proteasome inhibitor, MG-132. The size and context of protein aggregates varied highly. Hsp70 and ubiquitine 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. Proteasome inhibitor increased cell death in ARPE-19 cells.

Conclusion This study reveals that ubiquitin-proteasome pathway is an important way to control protein turnover in the RPE cells. In addition, Hsp90 inhibitor reflects to the cytoplasmic protein aggregation in ARPE-19 cells.

■ 205

Methylglyoxal destabilizes HIF-1 α by an ubiquitin-proteasome-dependent pathway in retinal pigment epithelial cells

BENTO CA (1, 2), PEREIRA P (1)

(1) Institute of Biomedical Research in Light and Image (IBILI) - Ophthalmology Center, Coimbra

(2) Center for Neurosciences and Cell Biology - University of Coimbra, Coimbra

Purpose Hyperglycaemia and ischemia are cross coupling pathophysiological events associated to a variety of eye diseases, including diabetic retinopathy. This study is designed to establish the mechanisms whereby HIF-1 (hypoxia-inducible factor 1), which is a transcription factor that triggers protective and adaptive mechanisms for cell survival under hypoxia, is regulated under low oxygen tensions and hyperglycaemia in human retinal pigment epithelial cells.

Methods ARPE-19 cells were treated with CoCl₂, low oxygen and MG132, simultaneously with methylglyoxal (MGX) or high glucose. Treatments were also performed in cells transfected with pcDNA3-HIF1 α (wt) and pcDNA3-HIF1 α (P402A; P564A). Whole cell lysates were assayed by immunoblot for HIF-1 α expression. HIF-1 α was also immunoprecipitated and probed against ubiquitin conjugates.

Results The results obtained indicate that MGX, a product of glucose metabolism, and hyperglycaemia revert the accumulation of HIF-1 α induced by hypoxia and proteasome inhibitors, in a time- and dose-dependent manner. Data further indicates that destabilization of HIF-1 α requires prior ubiquitinylation of this subunit and subsequent degradation by the proteasome. Significantly, the MGX-dependent destabilization of HIF-1 α appears to be independent of P402 and P564 hydroxylation, the common mechanism involved in ubiquitinylation of HIF-1 α by the E3 Von Hippel-Lindau protein.

Conclusion MGX stimulates degradation of HIF-1 α by a non-canonical mechanism that is independent of prolyl-hydroxylation. The increased production of MGX associated with diabetes can compromise the ability of the cells to adapt to low oxygen tensions thus leading to cell damage and retinal dysfunction.

■ 206

Cyclooxygenase-2 gene expression and immunolocalization in human epiretinal membranes

SCHUMANN RG (1), BAUER C (2), STRAUSS R (1), YU AL (1), GANDORFER A (1), KAMPIK A (1), WELGE-LISSEN U (1)

(1) Ophthalmology, Ludwig-Maximilians-University, Munich

(2) Clinical Pharmacology, Ludwig-Maximilians-University, Munich

Purpose Cyclooxygenase-2 (COX-2) known for its enzymatic activity in inflammation and ischemia has been shown to modulate cell growth and angiogenesis. We investigated the involvement of COX-2 in epiretinal membrane (ERM) formation of eyes with proliferative vitreoretinopathy (PVR), proliferative diabetic retinopathy (PDR), and idiopathic epiretinal membranes (IERMs).

Methods COX-2 gene expression was examined using quantitative RT-PCR analysis of ERM samples harvested from eyes that underwent standard vitrectomy for PVR, PDR and IERMs. Immunohistochemical staining of COX-2 proteins was performed on ten cryofixed samples of all groups by the avidine-biotin method.

Results COX-2 mRNA expression levels were markedly higher in PVR (6 fold) and PDR (3.5 fold) membranes compared to IERMs. Immunohistochemical analysis showed a strong COX-2 protein staining in cellular elements of PVR and PDR membranes, whereas in IERMs only a weak staining was observed.

Conclusion COX-2 gene expression and protein localization in epiretinal membranes of eyes with PVR and PDR suggest an important role of inflammatory and ischemic processes in the pathogenesis of these retinopathies. In contrast, COX-2 appears to be less involved in the formation of IERMs.

■ 207

Quantitative astroglial changes in an experimental model of hypercholesterolemia; variations after normalization of cholesterol blood values induced by a normal diet

RAMIREZ AI (1), SALAZAR JJ (1), DE HOZ R (1), ROJAS B (1), RUIZ E (2), TEJERINA T (2), TRIVINO A (1), RAMIREZ JM (1)

(1) Instituto de Investigaciones Oftalmológicas Ramón Castroviejo. UCM, Madrid

(2) Department of Pharmacology, School of Medicine, UCM, Madrid

Purpose To evaluate quantitative changes in retinal astrocytes in a rabbit model of hypercholesterolemia, as well as their changes after a period of normal blood-cholesterol values induced by a normal diet.

Methods New Zealand rabbits were divided into three groups: G0, fed a standard diet; G1A, fed a 0.5% cholesterol-enriched diet for 8 months; and G1B, fed a 0.5% cholesterol-enriched diet for 8 months, and then with a standard diet for another 6 months. Retinal whole-mounts were processed by immunohistochemistry (anti-GFAP). The total area occupied by astrocytes in the medullated nerve fibre region (MNR), the area occupied by astrocytes associated with the nerve-fibre bundles (AANFB) and the area occupied by perivascular astrocytes (PVA) were studied by computer-assisted morphometric analysis.

Results The area occupied by the AANFB and by PVA in G1A and G1B was significantly lower than in controls. However, no significant differences in PVA were found between G1A and G1B. In G1B, type I PVA was absent and replaced by hypertrophic type II cells.

Conclusion The maintenance of the area occupied by the PVA in G1B animals could be due to the hyperplasia of Type II PVA as an attempt to compensate for the loss of Type I PVA. This cell proliferation is presumably a response to the sustained retinal ischemia suffered undergone by G1B rabbits despite of normalization of cholesterol levels.

■ 208

Preservation of retinal anatomy and synaptic connectivity following preventive subretinal injections of Schwann cells in the RCS rat

PINILLA I (1), CUENCA N (2), SAUVE Y (3), MARTINEZ-NAVARRETE G (2), BORQUE E (1), LUND RD (4)

(1) Ophthalmology, Hospital Universitario Miguel Servet, Zaragoza

(2) Biotechnology, Alicante

(3) Ophthalmology and Physiology, Edmonton

(4) Ophthalmology and Visual Science, Salt Lake City

Purpose To examine the effect of preventive subretinal Schwann cell injections in the RCS rat on the outer retina and synaptic connectivity between photoreceptors and their target neurons.

Methods Twelve rats received subretinal injections of a human Schwann cells at age P22; they were sacrificed at P70 (n=4), P90 (n=4) and P120 (n=4). Controls consisted of 9 unoperated rats sacrificed at the same time points (n=3) and 3 non-dystrophic RCS rats at P30. All animals were immunosuppressed with oral cyclosporine. The outer plexiform layer (OPL), outer nuclear layer (ONL), inner nuclear layer (INL) and inner plexiform layer (IPL) were studied immunohistochemically and retinal function examined with the electroretinogram (ERG).

Results In areas preserved by injected cells, maintained organization was evident in all the studied layers. Photoreceptors were clearly preserved. 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. The number of cells and their 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, always less frequent than in dystrophic untreated eyes. ERG recordings showed at best the persistence of both a- and b-waves at P70 and P90, which reflected the level of anatomical preservation achieved in treated animals.

Conclusion Photoreceptor rescue following Schwann cell injections is accompanied with the maintenance of synaptic connectivity and preservation of ERG.

■ 209

Expression of lumican and keratocan in normal and keratoconus cornea

AKHTAR S (1), BRON AJ (2), KERR BC (3), CATERSON B (3), HUGHES C (3), MEEK KM (1)

(1) School of Optometry and Vision Sciences, Cardiff University, Cardiff

(2) Nuffield Laboratory of Ophthalmology, Oxford University, Oxford

(3) School of Biosciences, Cardiff University, Cardiff

Purpose Lumican and keratocan are members of small leucine-rich proteoglycan (SLRP) and major keratan sulphate (KS) proteoglycan in corneal stroma. The absence of lumican leads to formation of cloudy cornea due to alteration in collagen fibril diameter and fibril spacing. Absence of keratocan exhibits thin but transparent cornea. In this study we investigated the distribution of lumican and keratocan in normal and keratoconus corneas.

Methods Four normal and four severe keratoconus corneas were used for the study. Monoclonal antibodies against lumican (1C3) and keratocan (11C3) were used with western blot and immunogold electron microscopy to quantify the labelling of lumican and keratocan.

Results Western blot studies showed that in normal cornea, lumican reacted strongly at 50kDa where as keratocan did not show any reactivity. In keratoconus cornea, both lumican and keratocan showed strong reactivity at 25kDa. Immunogold microscopy showed that labelling of lumican antibody was less in keratoconus cornea compared to normal cornea. Labelling of keratocan was also observed in keratoconus cornea but not observed in normal cornea.

Conclusion The results suggest that decrease in lumican expression in keratoconus cornea may leads to irregular distribution of collagen fibril diameters which is reported elsewhere.

■ 210

Corneal sensitivity and corneal nerve morphology in diabetic patients

FRUSCHELLI M, MARTONE G, HAKA G, TRAVERSIC, CAPOROSSI A
Ophthalmology and Neurosurgery University of Siena, Siena

Purpose Corneal confocal microscopy (CCM) gives detailed morphological features of corneal nerves. The purpose of this study was to evaluate whether the change of morphology of corneal nerve fibers corresponds to corneal sensitivity in diabetes.

Methods In this prospective study 60 eyes of 30 diabetic patients (stratified into mild, moderate and severe neuropathic groups) and 20 eyes of 10 control-normal subjects were examined by CCM and Cochet-Bonnet esthesiometer.

Results A correlation was found between the nervous morphology alterations demonstrated by CMM and the severity of diabetic neuropathy. In particular, the number and density of corneal nerve fibers decreased in diabetic patients.

Conclusion Damage of nerve fibers is responsible of peripheral neuropathy in diabetic patient. CCM is a rapid non-invasive *in vivo* technique that enables an important microscopic evaluation of corneal nerve alteration in diabetic patient. With analysis of corneal nerve density and morphology, a correlation has found between the alterations of corneal nerves and the severity of diabetic neuropathy.

■ 211

Where are the deposits in cornea verticillata in Fabry?

LANZL I (1), KOTLIAR KE (1), MAIER M (1), FIORE B (1), MESSMER EM (2)

(1) Eye Clinic, Technical University, Munich

(2) Ludwig Maximilians University, Munich

Purpose Fabry's disease is caused by a mutation in the X-linked galactosidase gene. Glycosphingolipids are deposited preferentially in vascular and reticuloendothelial tissue in this lysosomal storage disease. Pathognomonic ocular signs consist of cornea verticillata and conjunctival as well as retinal vascular abnormalities. Cornea verticillata in Fabry presents as a diffuse haze in the subepithelial layer initially with progression into cream-colored, whorl-shaped opacities of the cornea similar to changes found after prolonged treatment with chloroquine or amiodarone. The exact location of the deposits is unknown.

Methods *In vivo* confocal microscopy of both eyes was performed in a 22 years old female with Fabry's disease using the Heidelberg Retina Tomograph (HRT II) / Rostock Cornea Modul (RCM) in order to define the exact location of the observed opacities in the cornea.

Results Superficial cells, wing cells and basal epithelial cells of the cornea demonstrated highly reflective granular to amorphous intracellular inclusions. These hyperreflective changes were not uniform, but were distributed along distinct longitudinal areas. Hyperreflective changes were not observed extracellular. The epithelial basement membrane and Bowman's layer were unremarkable. Moreover, the corneal keratocytes and endothelial cells were unaffected.

Conclusion Among various types of corneal epithelial cells only basal cells have mitotic activity. Lysosomes are produced by the Golgi apparatus of the cell and obtain the enzymes, necessary for their digesting tasks from the rough endoplasmatic reticulum which is abundant in basal corneal epithelial cells. Therefore it is not surprising that the hyperreflectivity we observed in our case of Fabry disease is pronounced in that level.

■ 212

DACRIO – TC in the follow up of dacriocistorinostomy ab externo

PESCE GP, MESIANI MAZZACUVA FMM, CATALANO CC, RECHICHI MR,
CARNOVALE SCALZO G, PARDATSCHER KP, STANA CS, BOMBARDIERI CB
Magna Graecia University, Catanzaro

Purpose This study explains advantages of dacrio-TC for the follow-up of patients in which dacryocystorhinostomy ab externo was performed for lacrimal system stenosis.

Methods We performed 100 surgical procedures of dacryocystorhinostomy ab externo and then we followed patients at month 1, 6 and 12 by contrast dacrio-TC with lopamidol

Results We observed that after dacryocystorhinostomy procedure, rinostomy amplitude was reduced of 10% after 1 month, 30% after six months and 40% after 12 months.

Conclusion Dacrio- TC represents a valid, safely and reliable technique for the follow-up of patients treated with dacryocystorhinostomy for lacrimal system stenosis.

■ 213

Morgan's Transconjunctival Correction of Age-Related Lower Eyelid Entropion

GANDHI JS (1), ZIAHOSSEINI K (2), ODAT T (2), MANIK KS (3), MORGAN LH (2)
 (1) The Royal Eye Hospital, Manchester
 (2) Stepping Hill Hospital, Manchester
 (3) Leeds General Infirmary, Leeds

Purpose To describe and evaluate the long-term effectiveness of a new transconjunctival surgical operation devised for the repair of age-related lower eyelid entropion.

Methods A retrospective review of the case notes of 39 randomly selected patients (45 lower eyelids with age-related entropion) in whom this operation was performed between 1994 and 2002. All patients underwent a resection of the preseptal orbicularis oculi, a centrally placed excision of tarsconjunctival triangle (with the base towards the fornix), and a reinsertion of lower eyelid retractors. All operations were performed by the same surgeon (Laurence Morgan) at Stepping Hill Hospital in England, UK.

Results Out of 39 patients, there were 15 men and 24 women. The age range was 64 to 96 years (mean 84.3). Six patients underwent bilateral surgery. Nineteen eyelids had previous entropion repair using a different technique. At 6 weeks postoperatively 43 eyelids (98 %) had a reversal of entropion. The overall recurrence rate was 11 % (5 patients). Five patients (11 %) had minor complications such temporary notching of the lid edge or trichiasis.

Conclusion This surgical technique for the repair of lower eyelid age-related entropion is simple and takes relatively little operating time. In our unit we have found it to be effective and associated with a low complication rate. This procedure can be easily performed by the general ophthalmologist.

■ 215 / 2126

S. epidermidis: Friend or Foe in Dry Eye- Its Interaction with Goblet Cells

GRAHAM JE (1), MOORE JE (2), DARTT D (3), BEST R (2), ALARBI M (2),
 MCGIMPSEY S (2), DIABOLD Y (4), DOWNES CS (1), MOORE CBT (1),
 MOORE JE (5)
 (1) UU Coleraine, Coleraine
 (2) RVH Belfast, Belfast
 (3) Schepens Eye Research Institute, Boston
 (4) University of Valladolid, Valladolid
 (5) Belfast City Hospital, Belfast

Purpose Commensal bacteria may contribute to the pathogenesis of ocular conditions including dry eye. *S. epidermidis* is the most commonly isolated ocular surface bacteria. This study investigated the interaction of this bacterium with conjunctival epithelial and goblet cells.

Methods Primary conjunctival goblet cells were cultured as previously described by Shatos et al. *S. epidermidis* was isolated from the conjunctiva of both normal and dry eye subjects and a gentamicin invasion assay was performed with the conjunctival epithelial cell line IOBA-NHC and the primary goblet cells.

Results *S. epidermidis* isolated from the conjunctiva of both normal and dry eye subjects were internalised by conjunctival epithelial cells. In comparison when incubated with primary cultures of human goblet cells, internalisation only occurred with bacteria isolated from dry eye subjects. The genetically modified non-pathogenic *S. epidermidis* strain ATCC 12228 was not internalised by either cell type.

Conclusion Two *S. epidermidis* strains isolated from dry eye subjects, demonstrating the ability to invade primary human goblet cells in culture, would suggest such strains possess virulence mechanisms absent in ATCC 12228 and non dry eye isolates. Previous studies have suggested this invasive capacity of *S. epidermidis* may be linked to the increase in numbers of bacterium at a specific site. *S. epidermidis* is the most frequently isolated bacteria from the ocular surface and as a non-pathogenic commensal, may have the potential to become pathogenic when an increase in quantity is present such as that often observed in ocular conditions such as dry eye. This raises the question of whether invasion as a virulence factor may only be exerted by *S. epidermidis* through a quorum sensing mechanism when the bacterial population reaches a high concentration.

■ 214 / 2125

Antibiotic Resistances of Bacteria Identified in an Endophthalmitis Prospective Study

BRON AM (1), PASSEMARD M (1), LAFONTAINE PO (1), PECHINOT A (2),
 CORNUIT PL (3), ROUBEROL F (3), ROMANET JP (4), CREUZOT-GARCHER C (1),
 CHIQUET C (4)
 (1) Ophthalmology, Dijon
 (2) Department of Microbiology, University Hospital, Dijon
 (3) Ophthalmology, Lyon
 (4) Ophthalmology, Grenoble

Purpose To assess the antibiotic susceptibility of microorganisms identified in the vitreous and/or the aqueous humor of patients suffering from endophthalmitis.

Methods A multicenter prospective study was conducted from January 2004 to June 2005 to evaluate panbacterial Polymerase Chain Reaction (PCR) versus culture. We determined the antibiotic susceptibility of microorganisms identified by conventional identification (Culture in Brain Heart Infusion). Antibiotic resistance testing was performed using the disk diffusion technique in Mueller-Hinton agar or the VITEK2 system. We divided identified organisms into two groups, wild strain (WS) or with acquired antibiotic resistance (AAR).

Results We included 71 organisms from 64 endophthalmitis; twenty eight different microorganisms were identified. Among them, 84.5% were gram-positive Coccis ((staphylococcus 38/71, (53.5%), streptococcus 14/71, (19.7%). We found 53.5% (38/71) of WS organisms and 46.5% of AAR (33/71) with 7 multiresistant strains. Resistances to fluoroquinolone (FQ) were 13.2%, and vancomycin was still effective against 100% of all tested bacteria.

Conclusion This study about antibiotic resistances of microorganisms isolated in endophthalmitis finds an important part of them (46.5%) with AAR. Sensibilities to FQ are higher than in the literature. Micro-organism diversity is important and despite the predominance of gram-positive Coccis, a combination of intravitreous antibiotics is still recommended as the initial empiric treatment of endophthalmitis.

■ 216

Selective innate immunodeficiency in patients with severe herpesvirus retinitis?

BERGUA A (1), KITTAN N (2), HAUPT S (2), DONHAUSER N (2), KORN K (2),
 SCHMIDT B (2)
 (1) Department of Ophthalmology, University Erlangen-Nürnberg, Erlangen
 (2) Institute of Clinical and Molecular Virology, University Erlangen-Nürnberg,
 Erlangen

Purpose The first-line recognition of viruses and virus-infected cells is mediated via the innate immune system. An important component are plasmacytoid dendritic cells (PDC), which have been identified as major producers of type I interferons (IFN). Severe viral infections in non-immunosuppressed patients may result from selective defects in the innate recognition of these viruses.

Methods Peripheral blood mononuclear cells (PBMC) were isolated from EDTA-containing blood of healthy controls or patients with viral retinitis. PDC were purified from the PBMC population using a BDCA-4 Cell Isolation Kit. PDC were then challenged with herpes simplex type 1 (HSV-1), CpG-A (ODN 2336, Coley Pharmaceutical Group), and a synthetic TLR7 agonist (S27609, 3M Pharmaceuticals). Supernatants were analysed for the presence of IFN- α .

Results So far, 5 healthy controls and 3 patients have been included. One patient had experienced HSV-1 encephalitis, followed by HSV-1 retinitis 12 months later. The second patient had suffered from fulminant necrotizing uveitis caused by HSV-1, and the third patient experienced a varicella zoster virus (VZV)-associated retinitis. PDC of the control individuals readily responded to all stimuli with secretion of high IFN- α levels. In contrast, PDC obtained from the patients with HSV-1 retinitis did not respond to HSV challenge. PDC derived from the patient with VZV-retinitis produced IFN- α in response to HSV, but not CpG.

Conclusion Non-immunosuppressed patients who suffer from fulminant infection with HSV show defects in the induction of IFN- α production by PDC. Current studies are directed at identifying the block in IFN signaling.

■ 217

Detection of virulence factors in *Pseudomonas aeruginosa* strains isolated from contact lens-associated corneal ulcers

PINNA A (1), USAI D (2), ZANETTI S (2), BITTI A (3), PORCU F (3),
ZANETTI F (4), CARTA A (5), CARTA F (1)

(1) Institute of Ophthalmology, University of Sassari, Sassari

(2) Department of Biomedical Sciences, Section of Experimental and Clinical Microbiology, Sassari

(3) Laboratorio di Analisi Chimico-Cliniche e Microbiologiche, Ospedale Civile "A. Segni", Ozieri

(4) Department of Ophthalmology, Ospedale Civile "A. Segni", Ozieri

(5) Institute of Ophthalmology, University of Parma, Parma

Purpose To report on the microbiological findings of 8 *Pseudomonas aeruginosa* strains isolated from contact lens-associated corneal ulcers.

Methods Corneal scrapings from contact lens-related corneal ulcers were inoculated for culture. Identification and antibiotic susceptibility testing were performed using Vitek system (bioMerieux, France). *P. aeruginosa* isolates were analyzed for the presence of virulence properties, including production of alkaline protease, gelatinase, and elastase. The ability to adhere to human corneal epithelial cells (HCEC) and form biofilm on an abiotic surface was also investigated.

Results All the strains showed multiple antibiotic resistance (resistance to 4 or more antibiotics) and were able to produce alkaline protease and gelatinase, but not elastase. Adherence to HCEC cells was poor (0-15 bacteria/cell) in 5 cases and medium (16-60 bacteria/cell) in 3 cases. Biofilm production was weak in 5 cases and absent in the remaining 3.

Conclusion Results suggest that the ability to produce alkaline protease and gelatinase may play a major role in the pathogenesis of *P. aeruginosa* keratitis associated with contact lens wear.

■ 218

Expression of Interleukin-6, Tumour Necrosis Factor-alpha and Nitric Oxides during Episodes of Ocular Toxoplasmosis in an HIV Patient

PRADA J (1), LIEKFELD A (2), BERGMANN F (3), GROBLUSCH MP (4)

(1) Medical Research Center, Charité-CVK, Berlin

(2) Dept. of Ophthalmology, Charité-CVK, Berlin

(3) Dept. of Infectiology, Charité-CVK, Berlin

(4) University of the Witwaterstrand, Infectious Diseases Unit, Johannesburg

Purpose At the present time, there are few reports concerning the expression of inflammatory markers and its possible role in patients with ocular toxoplasmosis. The aim of this study was to systematically analyse the expression of the inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha), as well as the accumulation of nitric oxides (NO) during ocular toxoplasmosis in an HIV patient.

Methods A 36-years-old male HIV-patient exhibited a *Toxoplasma gondii* infection with no signs of encephalitis, but with different episodes of ocular toxoplasmosis. Plasma samples from this patient were collected and systematically analysed during a time period of more than 4 years. IL-6 and TNF-alpha bioactivities were analysed by the B9 and the WEHI164 bioassays, respectively. NO and neopterin levels were further evaluated by a compensated Griess reaction and EIA/ELISA, respectively.

Results The highest recorded IL-6, TNF-alpha and NO levels were always detected during the observed episodes of ocular toxoplasmosis. Especially, the observed highest levels of IL-6 were statistically significant ($P = 0.0049$) during ocular toxoplasmosis, when compared to the rest of recorded IL-6 values.

Conclusion The analysed inflammatory markers seem to be especially up-regulated in plasma during the recorded episodes of ocular toxoplasmosis. The observed high levels of IL-6, as well as those of TNF-alpha and NO may play a protective role for the host against active *T. gondii* parasites.

■ 219

Ocular Presentation of Syphilis with Co-incident HIV Infection

WRIDE NK, PANDIT RJ

Department of Ophthalmology, Royal Victoria Infirmary, Newcastle-upon-Tyne

Purpose A case of previously undetected Syphilis and Human Immunodeficiency Virus (HIV-1) infection presenting with ocular signs.

Methods This patient presented with a 2 day history of painless, reduced vision in his left eye to his local eye unit. He had previously been tested positive for Hepatitis B. He denied intravenous drug use but was bisexual. On examination his visual acuity was 6/9 in the right eye and 'counting fingers' in the left. He was noted to have a localised placoid posterior chorioretinitis and a pan-uveitis. He was treated initially with oral steroids with subsequent worsening of the intraocular inflammation and the onset of pain.

Results He was referred to our department where he was admitted for investigation. He was diagnosed with HIV-1 infection and syphilis from venous blood samples. He was treated with high dose intravenous penicillin. He did not require anti-retroviral therapy. The uveitis responded dramatically over a four week period with improvement in vision to 6/9 in the affected eye. After 6 months of follow-up there has been no reactivation of his uveitis.

Conclusion The manifestations of ocular syphilis are numerous and well documented. Although a common cause of uveitis in the early twentieth century, syphilis is now rarely seen other than in high risk groups. This case was in part informative due to the prolonged history prior to the eventual diagnosis and treatment. The effects of steroid and subsequent deterioration of the uveitis are of interest as is the remarkable recovery in visual function with penicillin treatment alone. This case demonstrates the role that the ophthalmologist can play in the detection of infectious disease.

■ 220

Humira / Adalimumab therapy in non-infectious posterior segment uveitis

MISSOTTEN T (1), VAN LAAR JAM (2), VAN DAELE PL (2), VAN DER LOOS T (3), BAARSMA GS (1), VAN HAGEN PM (2)

(1) Uveitis dept Rotterdam Eye Hospital, Rotterdam

(2) Immunology dept Erasmus University, Rotterdam

(3) Internal medicine dept Rotterdam Eye Hospital, Rotterdam

Purpose In recent years, TNF- α blocking agents have gained popularity in the treatment of severe non-infectious uveitis. Infliximab-Remicade appears to be a truly beneficial drug for the treatment of severe uveitis, and Etanercept-Embrel has less favorable results. Humira/Adalimumab has little reported data describing its efficacy in uveitis.

Methods Non-randomised non-controlled study. In 12 patients with severe panuveitis, Humira / Adalimumab was used as a steroid-sparing (4) or mono (8) therapy. Patients received 40 mg Humira by subcutaneous injections fortnightly. Data was analysed for safety, visual outcome and disease activity parameters.

Results Patients included in this trial had a history of 10 ± 4 years of uveitis and 5 ± 1 treatment combinations prior to Humira administration. Reduction of disease activity was noted in 11 of 12 patients, usually starting after 2 months of Humira administration. At 6 months, 89% of eyes were quiescent, and visual acuity improved in 80% of eyes. In one patient Humira was stopped as the uveitis did not improve. In 3 patients a cutaneous reaction was noted after 6-9 months of treatment, needing further arrest of treatment in 2 patients.

Conclusion In this non-randomised non-controlled study, Humira/Adalimumab has visual acuity and activity outcomes comparable to Infliximab/Remicade. Cutaneous reactions are the most frequent side-effect. Humira/ Adalimumab has a favorable (subcutaneous) route of administration compared to previous anti-TNF- α regimen. A prospective controlled trial should establish its role in severe non-responsive uveitis.

■ 221

OCT – SLO findings in a case of toxoplasma chorioretinitis complicated by epimacular membrane: a new diagnostic tool

PICCIIRILLO V (1), RAO V (2), DI NUZZO A (2), FORTE R (3), DE CRECCHIO G (3), SAVASTANO S (1), SAVASTANO MC (4), MOSCATO B (5), ROSEN R (6)

(1) Eye Department, Civilian Hospital Santa Scolastica, Cassino - FR

(2) Eye Department Civilian Hospital of Maddaloni, Maddaloni - CE

(3) Eye Department, University Federico II, Naples

(4) Eye Department, Catholic University, Rome

(5) Radiology Department, Civilian Hospital G Rummo, Benevento

(6) New York Eye and Ear Infirmary, New York

Purpose To describe the latest OCT –SLO diagnostic technique and its application in vitreoretinal and chorioretinal interfaces evaluation in a case of toxoplasma chorioretinitis complicated by epimacular membrane.

Methods A young woman (24 yo) suffering for toxoplasma chorioretinitis in the right eye (2 foci at the posterior pole, in iuxtafoveal area), stable since February 2004, therapy-free, applied to the Primary Care Unit complaining of visual loss and metamorphopsias in the right eye since 20 days. Complete ophthalmic examination, fluorescein angiography and OCT – SLO were performed.

Results Best corrected visual acuity: 20/40 in the right eye (vs 20/25 of 2004), 20/20 in the left eye. IOP: 17 mmHg in both eyes. Fundoscopic examination did not reveal modifications in size and pigmentation of chorioretinal scars (compared with 2004' photos) but a light macular swelling and abnormal inner limiting membrane reflex. No signs of reactivation were seen using fluorescein angiography. OCT-SLO evaluation of the macular area revealed an epiretinal membrane sticking to both neurosensory retina above chorioretinal scars and foveolar area causing light tractive intraretinal oedema.

Conclusion Epimacular membranes may complicate iuxtafoveal chorioretinitis despite no clinical signs of toxoplasma reactivation. The OCT-SLO diagnostic technique, through coronal imaging of the posterior pole, allows an high resolution evaluation of vitreoretinal and chorioretinal interfaces useful in pathophysiological understanding of toxoplasma chorioretinitis complications.

■ 222

Ocular hypotony in Fuchs heterochromic cyclitis: case report

PARISE S, PENDINO V, VARANO L, SCORCIA GB, SCORCIA V,

CARNOVALE SCALZO G, SCORCIA G

Eye Clinic Magna Graecia University, Catanzaro

Purpose We report a case of hypotony in Fuchs heterochromic cyclitis (FHC). Our patient, a 30-years old male, show since 5 years all FHC signs like keratic precipitates, anterior chamber reaction, vitreous opacities, iris stromal atrophy with heterochromia and cataract, but he didn't develop glaucoma. Because in cases of ocular hypotony, the cause is related to ciliary bodies pathologies in 80%, we want investigate their ultrasonographic morphological alteration in a patient with chronic iridocyclitis.

Methods We have performed an ultrabiomicroscopy (13.20 Mhz) to obtain real-time images of the subsurface structures of the anterior portion of the living eye at microscopic resolution. We have evaluated morphological and anatomic alterations of ciliary bodies by ultrabiomicroscopy analysis and compared this data to another performed 5 years ago in early fase of disease.

Results Ultrasonographic assessment revealed morphometric alteration in ciliary bodies, that probably causes hypotony whereas it could be hyperthyroid.

Conclusion A chronic inflammatory activity in eye anterior segment can cause a morphofunctional alteration of ciliary bodies with hypotony in that patients with FHC that don't develop glaucoma.

■ 223

Secondary open-angle glaucoma caused by sarcoidosis: a rare case

CATALANO CC, PESCE GP, RECHICHI MR, FRATTO MF,

MESIANI MAZZACUVA FMM, SCORCIA G

Magna Graecia University, Catanzaro

Purpose we presented a rare case of secondary open-angle glaucoma due to uveitis post-systemic sarcoidosis

Methods We examined a patient 42 year-old, female that referred a pulmonary sarcoidosis diagnosis in 1988, documented by chest X-ray, gallium 67 scintigraphy, pulmonary biopsy, ACE enzyme, HLA-B27 positivity and also iridocyclitis episode in OS in 1998 associated with ocular hypertension. We performed a full ophthalmological examination: BCVA, slit lamp biomicroscopy, gonioscopy, IOP measurement, fundus oculi, HRT, GDx, UBM, visual field, HRF, OCT and then we repeated chest X-ray and complete immunological assay. We followed the patient for 18 months by BCVA evaluation, slit lamp biomicroscopy, IOP measurement, visual field, ophthalmoscopy and sarcoidosis activity index evaluation.

Results We observed signs of ocular sarcoidosis: Koeppe's nodules, corneal endothelial deposits, pigmentary dispersion on anterior capsule of lens, severe visual field defects, cup/disc alteration. During 18 months of follow-up, with our therapies, we not observed worsening of systemic and ocular disease documented by serological tests or ocular disease.

Conclusion During 18 months follow-up we not observed reactivation of systemic disease documented by serological tests or ocular disease.

■ 224

Case Series on Scleritis in Newcastle

VELUSAMIP (1), SANDHUS (2), PANDIT RJ (2)

(1) Queen Mary's Hospital, Sidcup

(2) Royal Victoria Infirmary, Newcastle upon Tyne

Purpose Review of the experience of patients with scleritis in Newcastle upon Tyne, UK.

Methods Retrospective case note review examining the characteristics of new patients referred to ocular inflammation clinic.

Results Over an 18 month period, 26 patients with mean age = 47.5 years (range 21 – 67). They were followed up 3 – 18 months. Ocular pain and globe redness were presenting symptoms in all patients, although severe pain was reported in only 46.2% of cases. Average duration of symptoms prior to the first visit was 14.2 days (95% CI: 6.7 to 26.3). Average time between initial presentation and diagnosis 17.2 days (95% CI: 5.9 to 28.9). Average total duration of uncontrolled symptoms was 43.1 days (95% CI: 31.2 to 54.9). Diagnosis at first visit was scleritis in 46.2%, episcleritis in 34.6%, viral conjunctivitis in 7.7%, and other diagnosis in 11.5%. Scleritis was often inadequately controlled with topical therapy and required treatment with oral steroids (76.9%), second-line immunosuppression (50.0%) and IV methyl-prednisolone (34.6%). Associated systemic disease was present in 30.8% of cases, with scleritis being the first manifestation in 37.5%.

Conclusion Scleritis is commonly misdiagnosed. The relatively long duration of uncontrolled symptoms (43.1 days) are partially accounted for by delay in presentation to an ophthalmologist and subsequent delay of adequate treatment. A large proportion requires oral steroid or 2nd line immunosuppression to achieve symptom control. Investigation for systemic association is warranted, with scleritis often being the first manifestation.

■ 225

Agitation of contact lenses by the use of the Complete Rapid Care Cleaning Device

NTAMPOS K (1), NATSIS K (2), KARAMPATAKIS V (1), GOULIA O (1),

ANTONIADIS M (1), SAMANIDOU E (1), AMIRIDIS I (2)

(1) Experimental Ophthalmology, Aristotle University, Thessaloniki

(2) Laboratory of Anatomy-Microscopy Department, Aristotle University, Thessaloniki

Purpose To determine the efficacy of agitation of the Complete Rapid Care Cleaning Device in soft contact lens care.

Methods We examined 50 soft contact lenses stored and preserved by their users in various cleaning solutions and after the conventional lenses had been used for almost one year and the monthlies for more than one month. Each lens was observed and photographed under the light microscope. Then the lenses were placed in the Rapid Care device with Complete Moisture Plus solution and agitated for 1 min. The Complete solution was renewed and a second agitation was performed for 1 min. Finally the lenses were observed again under the light microscope.

Results Under the light microscope, the lenses presented various surface deposits. After agitation with the Complete Rapid Care Cleaning Device, the vast majority of these surface deposits were removed and the lenses were surprisingly clearer. The agitation did not affect the integrity of the lenses.

Conclusion Various superficial deposits (debris, salts, organic substances, foreign bodies and potential micro-organisms) may produce irritation and discomfort to the wearers. Agitation of contact lenses by the use of the Complete Rapid Care Cleaning Device removes the surface deposits and does not affect the integrity of the lenses.

■ 226

Silencing of nitric oxide production by siRNA, application for corneal transplantation

NEIWIRTH A (1), FRIC J (1), JIRSOVA K (2), FILIPEC M (2), HOLAN V (1)

(1) Faculty of Natural Sciences, Charles University, Prague

(2) Laboratory and Ocular Tissue Bank, General Teaching Hospital and Charles University, Prague

Purpose Inhibition of nitric oxide (NO) production in primary mouse macrophages and macrophage cell line P388D1 by siRNA targeting inducible NO synthase (iNOS) gene. Selected siRNA molecule will be used for in vivo application with the main purpose to inhibit cornea allograft rejection.

Methods Peritoneal mouse macrophages and P388D1 macrophage cell line were used for all experiments. Liposome/amine based siRNA transient transfection was used to silence iNOS gene expression in LPS/IFN- γ stimulated cells. Gene expression was determined by Real-time RT-PCR, ELISA and Western Blot. Nitric oxide was measured by Griess reaction. MTT test was applied to evaluate toxicity of the compounds. Changes in phenotype of the examined cells were analyzed by FACS.

Results Polyamine based siRNA transfection resulted in a specific iNOS silencing as was documented on the level of mRNA and protein synthesis. In addition, iNOS gene suppression was strongly associated with decreased level of NO in cell culture medium. Expression of genes for several other inflammatory cytokines or arginase I were not influenced by the treatment. MTT test did not reveal significant cell mortality after siRNA-amine treatment. Fluorescent labeled negative siRNA, used as another control, which have comparable transfection efficiency, also did not induce nonspecific silencing effect.

Conclusion Our data indicate that siRNA effect in primary macrophages and cell line was target specific. We showed that siRNA technology can manage NO production in vitro and hypothetically can be used to inhibit NO production after corneal transplantation. However, delivery strategy, the main obstacle for in vivo application still remains to be overcome.

■ 227 / 2344

Evolution of Ultraviolet Radiation-B (UVR-B)-Induced Cataract in the Pigmented Guinea Pig

MODY VC, KAKARM, LÖFGREN S, AYALA M, MEYERL, DONG X, WANG J, BUCHT C, SÖDERBERG PG

Karolinska Institute/St. Erik's Eye Hospital, Stockholm

Purpose To investigate the short-term development of cataracts after acute exposure to ultraviolet radiation-B (UVR-B) in the pigmented guinea pig.

Methods Twenty-four female pigmented guinea pigs, five to eight weeks of age, were exposed unilaterally to 80 kJ/m² UVR-B under anesthesia *in vivo* for sixty minutes. The guinea pigs were euthanized after post-exposure intervals of 1, 2, 4, or 8 days. Each lens was extracted and photographed. The development of forward light scattering for the lens was used to quantify the amount of cataract as expressed in transformed equivalent diazepam concentration units (tEDC).

Results All non-exposed lenses were devoid of cataract. All exposed lenses developed anterior subcapsular opacities at 1 day after UVR-B exposure. The surface area of the opacities decreased from 2 days to 8 days after exposure. Exposed lenses scattered more light than the corresponding contralateral lenses for the animals of the four groups. The mean differences \pm 95% confidence intervals in forward light scattering between the exposed and non-exposed lenses for each group in tEDC were: 0.052 \pm 0.036 [1 day]; 0.034 \pm 0.018 [2 days]; 0.041 \pm 0.024 [4 days]; and 0.044 \pm 0.018 [8 days]. A maximum amount of forward light scattering in the exposed lens developed at one day post-UVR-B-exposure and was still present up to eight days after exposure.

Conclusion Anterior subcapsular cataract develops one day after exposure to UVR-B in the pigmented guinea pig. Maximum intensity of forward light scattering develops one day after exposure to UVR-B and remains constant up to eight days after exposure. One day is an appropriate time interval for study of UVR-B safety limit in the pigmented guinea pig.

■ 228 / 2345

A Phacoemulsification Anticipation of Difficulty (PAD): Anterior chamber depth predicts difficulty of surgery

SIDDQUI S (1), SOLEBO L (1), HAYWARD JM (1), KERR-MUIR M (2), GALE RP (1)

(1) Department of Ophthalmology, York Hospital, York

(2) Department of Ophthalmology, Addenbrookes Hospital, Cambridge

Purpose The training of ophthalmologists to perform cataract surgery is becoming an increasingly contentious issue. Studies have shown that 37% of cataract operations are performed by those at specialist registrar level or below. It is imperative that cases of appropriate difficulty are selected throughout the training of cataract surgeons. We designed a 10-point user-friendly scale to anticipate difficulty of phacoemulsification cataract surgery at pre-operative assessment for use as a tool for the trainee ophthalmologist.

Methods 84 patients were studied over a 4-month period following COREC and research and development approval. Data was collected using a standardised preoperative scale. Perceived post-operative difficulty was scored on a visual analogue scale by 3 experienced consultant surgeons, blinded to the pre-operative score.

Results There was no difference in inter-observer variability for pre-operative scoring ($p < 0.05$). A strong correlation between the pre-operative grading of the anterior chamber depth (either deep or shallow) and perceived difficulty of surgery is demonstrated ($p < 0.05$). This indicated that AC depth was the most powerful predictor intraoperative difficulty.

Conclusion The PAD scale ensures thought is given to the anticipated difficulty of cataract surgery prior to case selection for ophthalmologists in training.

■ 229 / 2346

Cataract and inflammation: a new drug delivery system

EPERON S (1), BOSSY-NOBS L (2), GUEX-CROISIER Y (1), PETROPOULOS IK (1), GURNY R (2)

(1) Jules Gonin Eye Hospital, University, Lausanne

(2) Department of Pharmaceutics and Biopharmaceutics, University, Geneva

Purpose Cataract surgery is often performed in patients suffering from other pathologies, which imply high doses of steroids via oral or injection route. Moderate efficacy, risks and secondary effects are often observed. Thus it is of great importance to develop a biodegradable drug delivery system (DDS) associated to the artificial intraocular lens (IOL).

Methods DDS were manufactured using poly(D,L-lactide-co-glycolide) as matrix. The amount of triamcinolone acetonide (TA) loaded in DDS was evaluated as well as the release profile of TA. Cataract surgery was performed on the right eye of pigmented rabbits followed by DDS insertion. Inflammation parameters were: i) the clinical score (sum of hyperemia, chemosis, edema and secretion, each scored from 0 to 3), ii) the number of inflammatory cells and iii) the protein concentration in aqueous humor (AH).

Results The DDS weighed 2.94 ± 0.08 mg and measured 2.1 ± 0.2 mm. The loading capacity was approximately of 338 ± 7 microg of TA per mg of polymer. Comparisons were made between rabbits wearing i) no DDS, ii) unloaded DDS, iii) loaded DDS, iv) two loaded DDS. The in vivo investigations showed a good ocular biocompatibility. Loaded DDS could significantly reduce ocular inflammation induced by surgery, especially concentration of inflammatory proteins within AH.

Conclusion The results suggest that our type of DDS loaded with TA would be a promising system for the delivery of steroids to reduce ocular inflammation.

■ 230 / 2347

Ocular Anaesthetic Scoring System: Does the use of hyaluronidase in ocular anaesthesia improve patient satisfaction?

CEHAJIC J (1, 2), WEARNE IM (2)

(1) West Kent Eye Centre, Princess Royal University Hospital, Bromley Hospitals NHS Trust, London

(2) Eastbourne District General Hospital, Eastbourne

Purpose To use Ocular Anaesthetic Scoring System (OASS) to compare effectiveness of anaesthetic techniques and to determine whether this correlates with patient satisfaction of non-topical anaesthesia during cataract surgery.

Methods We developed OASS as a quick means of assessing non-topical anaesthesia. A score between 0 (poor) and 14 (excellent) is obtained using objective (degree of ptosis, akinesis and orbicularis function) and subjective measures (digital spear pressure at limbus and topical anaesthetic sting). 75 patients were randomised to receive either sub-Tenon's (ST) or peribulbar (PB) block. The ST group was further divided into a low (150 units) or high (300 units) level of hyaluronidase group. Anaesthesia was assessed immediately preoperatively using OASS. Postoperative patient satisfaction was determined by Visual Analogue Pain Scale (VAPS) and Iowa Satisfaction with Anaesthesia Scale (ISAS). Statistical analysis was performed using non-parametric tests.

Results Using OASS the ST approach achieved significantly better scores than the PB ($P<0.001$). Higher levels of hyaluronidase (300 units) were associated with significantly higher OASS scores ($P<0.001$). Spearman rank correlation showed that OASS correlates significantly with well-established subjective measures, VAPS (-0.60, $p<0.0001$) and ISAS (0.52, $p<0.0001$). The median satisfaction score was significantly higher in the ST than in the PB group and in the high level compared to the low level hyaluronidase group.

Conclusion The OASS has been shown to be an effective tool in assessing and comparing non-topical anaesthesia and techniques. Furthermore, a patient who has had a good block, as defined by OASS, reports significantly higher satisfaction scores with anaesthesia during cataract surgery.

■ 231

Quality of life and visual outcomes following cataract surgery in patients with early cataract

GILMOUR DF (1, 2), CACKETT P (2), DHILLON B (2)

(1) Leeds Teaching Hospitals, Leeds

(2) Princess Alexandra Eye Pavilion, Edinburgh

Purpose To assess visual function and quality of life in patients before and after having first eye cataract surgery for early cataract

Methods Twenty patients listed for cataract surgery with a best corrected visual acuity (BCVA) of 0.3 logmar (6/12 Snellen) in the worse eye were assessed preoperatively and 1 month postoperatively. The patients had no previous surgery in either eye and no other ocular pathology. Distance Logmar vision, contrast sensitivity (using pelli-robson chart) and glare disability (using pelli-robson chart with brightness acuity tester) were measured in the operation eye only and with both eyes open. Quality of life was assessed using the VF-14 questionnaire. Patients' ability to read a car number plate at 20.5 metres was also recorded.

Results There was a statistically significant improvement in all objective measures (i.e. BCVA, contrast sensitivity and glare disability in the operated eye and with both eyes). Subjective visual function as measured with VF-14 questionnaire scores also improved significantly. 6 patients could not read a car number plate to the legal requirement for driving (UK) before surgery, with all patients achieving this following surgery.

Conclusion Cataract surgery significantly improves quality of life and visual function in patients with early cataract and relatively good visual acuity.

■ 232

Long term visual outcome after surgery of cataract with hyperplastic primary vitreous- Fifteen year-experiences

AUTRATA R, HELMANOVÁ I, REHUREK J

Department of Pediatric Ophthalmology, Masaryk University Hospital, Brno

Purpose To evaluate the Visual outcome of cataract-PHPV eyes left aphakic with correction of contact lenses (CL) and compared it with those undergoing intraocular lens (IOL) implantation at the time of surgery during the first six months of their life.

Methods A total of 51 consecutive children with unilateral PHPV and cataract, treated and followed at our clinic between 1988-2001, were included in this retrospective study. The mean age at the time of surgery was 4.39 months (range: 2 to 6 months). The mean follow up time was 9.4 years (range: 5 to 17 years). Cataract extraction combined with vitrectomy and removal of embryonic remnants was performed in 24 eyes (46.9%) remained aphakic. Intraocular lenses were primary implanted in 27 eyes (53.1%) In 5 eyes (an anterior chamber IOL and in 22 eyes a posterior chamber IOL (sulcus or in-the-bag) was implanted. All children had patching of the dominant eye up to 70% of the waking time.

Results The best corrected final visual acuity (BCVA) of 6/15 or better was obtained in 32.6% eyes), 38.8% reached 6/21-6/60 BCVA, and 28.6% had a visual acuity of 3/60 to perception of light in the entire group. A higher rate of good visual acuity (6/15 or better) in children with pseudophakia (11 of 16 eyes, 68.7%) than in children with aphakia (5 of 16 eyes, 31.3%). A poor visual outcome (3/60 to perception of light) had 12 of 14 aphakic eyes and 2 of 14 pseudophakic eyes.

Conclusion Based on our results we suggest that PHPV eyes have a potential for favorable visual rehabilitation after surgery performed during the first six months after birth. Intraocular lens implantation may be a safe and useful option for the management of these children.

■ 233

Rationale for the development of a viscoelastic fitting all steps of cataract surgery: Rhexel®

RIGAL SASTOURNE JC (1), LEBRETON P (2)

(1) Ophthalmology, Val de Grace Hospital, Paris

(2) Laboratoire Corneal, Pringy

Purpose To develop a new OVD adapted to all steps of cataract surgery and to deliver the device in a single syringe. By combining in this device dispersive and cohesive properties that could be successively used throughout the operation.

Methods An experimental testing of different available OVD was conducted: the elastic modulus, the viscous modulus and the contact angle were compared. Based on these clinically validated devices we looked for a possible combination of a dispersive product with a viscous modulus higher than the elastic modulus, together with a large range of shear rate and a cohesive agent with opposite properties. Hypromellose known for its high surface tension, associated with significant spreading and reduced viscosity was used for dispersivity and hyaluronic acid for cohesivity. The new device was compared with a control group of two different devices (dispersive and cohesive).

Results The experimental results, simulating different surgical steps confirmed the expected different and successive rheological properties of the new OVD. A multicenter European study (France, Italy) was conducted in 150 consecutive operations to assess the dispersive effect, the cohesive properties, and the safety of the device. Endothelial protection, stability of the anterior chamber, safe implantation, a complete easy removal and absence of post-operative inflammatory reaction were noted in all cases.

Conclusion Rhéxel, the new OVD is a safe product, to be used as a single device in a majority of cataract operations.

■ 234

Comparison of higher order aberrations and contrast sensitivity after Verisyse phakic IOL, and Icare IOL

DIGHIERO PL (1), BRIAT B (2), AGUSSAN J (2), GICQUEL JJ (1)

(1) Jean Bernard University Hospital / Ophthalmology Department, Poitiers

(2) Clinique de l'Atlantique / Visiopole / Ophthalmology, La Rochelle

Purpose To evaluate higher order aberrations and contrast sensitivity after implantation of the Verisyse and the Icare phakic intraocular lens (IOL).

Methods In a prospective, non-randomized case series, Verisyse phakic IOL (AMO Inc, Mougins, France) was implanted in 9 eyes and Icare phakic IOL (AMO Inc, Mougins, France) was implanted in 9 other eyes. Ocular wavefront aberrations were measured 1 month postoperatively in all eyes using an IRX3 aberrometer (Imagine Eyes, France). Zernike expansions were computed up to the 6th order and normalized to a 4 mm pupil diameter. The wavefront performance of the two IOLs were compared using non parametric statistics.

Results The analysis revealed a difference in the distribution of spherical refraction between the two groups: this distribution was roughly centred on zero (emmetropia) with the Verisyse lens while it was shifted towards hyperopia, by less than 1 diopter, with the Icare lens. No statistical difference in higher-order RMS was found between the two IOLs. The average higher-order RMS, as well as vertical coma, was slightly higher with the Verisyse lens (N.S., Mann-Whitney U).

Conclusion Both IOLs has similar higher-order wavefront performance. Spherical refraction was slightly hyperopic in average with the Icare lens: this may likely be improved using refined power calculations.

■ 235

Bilateral Congenital Cataracts in Western Sweden: A Longitudinal Follow-up

SJÖSTRAND JB, NYSTRÖM A, MAGNUSSON GCB

Department of Ophthalmology, Göteborg University, Göteborg

Purpose In a previous study we showed a marked improvement in visual acuity before 10 years of age in a cohort of children with congenital cataracts operated before 36 weeks of age. The purpose of the present study was to describe visual acuity level and investigate visual acuity development after the age of 10 years, compared to Swedish normal populations from the literature.

Methods All children born in the county of Västra Götaland and Halland between 1980 and 1995 who were diagnosed with dense (n=20) or partial (n=28) bilateral congenital cataracts were included in a longitudinal prospective study. The best corrected visual acuity (BCVA) of the better eye was measured, expressed as decimal visual acuity.

Results In children operated before 36 weeks of age the mean BCVA at 10 years was 0.3 and 0.4 in total and partial cataracts, respectively. In children operated for partial cataract after one year the mean BCVA was 0.4. After 10 years the changes of BCVA were limited in all subgroups.

Conclusion The subnormal visual acuity of most children with congenital cataracts reached a plateau at 10 years of age. Therefore, the visual acuity at 10 years of age seems more predictive for final visual acuity than levels at 4 or 7 years of age commonly used for a normal population.

■ 236

Phakic refractive lens implantation to correct moderate to high hyperopia: 1 year follow-up

GIL-CAZORLA R (1, 2), FUENTES I (1), HERNANDEZ-VERDEJO JL (1, 2)

SANCHEZ JM (1), TEUS MA (1, 3, 4)

(1) Visum Corporacion Oftalmologica de Madrid, Madrid

(2) Escuela Universitaria de Optica - UCM, Madrid

(3) Universidad de Alcala, Alcala de Henares

(4) Hospital Princepe de Asturias, Alcala de Henares

Purpose To study the efficacy and safety of phakic refractive lens (PRL) implantation to correct high hyperopia.

Methods Prospective, observer-masked, interventional study. Inclusion criteria were 4 diopters (D) or more of cycloplegic hyperopia, best-corrected visual acuity (BCVA) of 20/40 or higher, anterior chamber depth of 3 mm or more, and mesopic pupil size 6 mm or smaller. The same surgeon implanted the PRL in all cases.

Results Sixteen eyes of nine patients were included in the study. The mean preoperative spherical equivalent refraction was $+5.65 \pm 1.41$ D (range, +3.25 to +5.75). The mean 1-year postoperative spherical equivalent refraction was 0.07 ± 0.43 D (range, -0.50 to 0.75). Fifteen eyes (93.75%) were within ± 0.5 D of emmetropia, and 16 eyes (100%) were within ± 1 D of emmetropia. The safety and efficacy indexes were 0.9 and 0.8, respectively. Eight eyes (50%) needed laser *in situ* keratomileusis to correct residual astigmatism. Five eyes (12%) lost one line of BCVA; none lost two lines or more of BCVA. The BCVA did not increase in any eye. No significant intraocular complications developed.

Conclusion PRL implantation to correct high hyperopia seems to be a safe and accurate procedure. A mild but significant loss in BCVA is anticipated.

■ 237

Clinical usefulness of visual evoked potentials in mild to moderate traumatic brain injury

MCKERRAL M (1), LACHAPELLE J (2)

(1) Psychology, University of Montréal, Québec

(2) Neurology-Neurosurgery, McGill University, Québec

Purpose To evaluate, using electrophysiological methods, the effects of a mild or moderate traumatic brain injury (TBI) on different complexity levels of visual information processing in adults receiving outpatient rehabilitation interventions.

Methods Three visual evoked potential (VEP) paradigms were separately administered to 23 participants (mean age 37.5 yrs) having sustained a TBI (15 mild, 8 moderate) and to 20 normal controls (mean age 29.6 yrs): 1) pattern-reversal VEPs (prVEP, P1): low-level condition, obtained from Oz, checks 0.5 deg., 90% contrast, reversal rate 0.5 Hz; 2) texture-segregation VEPs (tsVEP, N2): intermediate condition, recorded from Oz, line gradients 0.1 degree, 90% contrast, reversal rate 1 Hz; 3) cognitive VEPs (ERP, P3): higher-level condition, obtained from Pz, oddball paradigm, frequent stimulus 0.5 deg. check, rare stimulus 1.5 deg. check, 30% contrast, 0.3 sec. onset, 1.7 sec. offset.

Results Amplitude and latency of prVEPs obtained from TBI participants were not significantly ($p>0.05$) different from normal controls. TsVEPs were significantly ($p<0.01$) delayed in latency in TBI, but there were no amplitude differences. ERPs obtained from TBI subjects were significantly ($p<0.05$) reduced in amplitude, as well as significantly ($p<0.001$) delayed in latency compared to controls.

Conclusion Low level visual processing appears to be spared in our TBI group. However, TBI individuals show increasing visual processing deficits with increasing stimulus/task complexity. Such functional alterations are in line with known pathophysiology of milder forms of TBI and the methodologies used thus show promise in their ability to identify functional abnormalities which go undiagnosed with conventional neuroimaging techniques.

■ 238

VEPs results in children with meningitis

PILECKI W (1), SOBIESZCZANSKA M (1), SALOMON E (2), JANOWA A (2), KOLEDA P (1)

(1) Department of Pathophysiology, Wrocław Medical University, Wrocław

(2) Department of Physiology, Wrocław Medical University, Wrocław

Purpose Cortical blindness in children can occur after purulent meningitis. Aim of the study was to evaluate with VEPs an effect of purulent meningitis on visual analyzer in children at various age.

Methods Out of 41 children with previous purulent meningitis, 17 were tested 2 or 3 times. The children age was considered as corrected age, a sum of pregnancy duration and postnatal age. At the first VEPs examination, the patients' age varied a lot, 1–463 weeks of life. The next recordings were performed after 10–106 weeks, and the patients' age ranged 15–519 weeks. VEPs examinations were registered with 1 Hz flash stimulus and then morphology and P100 peak latency were analyzed.

Results The VEPs results from 14 of 41 examined children could be assessed as normal (34.1%), with typical waveforms and P100 latency within the lab limits. The VEPs tracings from the left 27 patients (65.9%) were evaluated as abnormal (illegible tracings in 13, delayed P100 in 4, reverse peaks' polarization in 8 and interhemispheric asymmetry in 2). Analysis of the VEPs results obtained in the particular age subgroups revealed: the normal VEPs were found in the oldest children (mean age: 186 weeks); the children demonstrating the slightest VEPs changes, i.e. P100 latency delay, were insignificantly younger (mean age: 154 weeks); the patients with serious VEPs abnormalities were significantly younger (mean age: 63 weeks, $p<0.05$).

Conclusion The results suggest that the younger are the children affected with meningitis, the more probable is an occurrence of disturbances of activation transmission within the visual pathway.

■ 239

The MVEP - problems and benefits of the method

GRZYBOWSKI AE (1, 2), HULEWICZ A (3)

(1) Electrophysiology Department, City Hospital „Nowe Miasto”, Poznań

(2) Department of History of Medicine, Poznań University of Medical Sciences, Poznań

(3) Division of Metrology, Institute of Electronics and Telecommunications, Poznań University of Technology, Poznań

Purpose The main limitation of the standard VEP technique is that it contains the averaged response from the whole stimulated retina and optic nerve, and does not provide a topographical measure. The multifocal VEP (MVEP) provide independent responses from multiple areas of the visual field simultaneously. However, one of the main problems reported in the literature is the non-stability of the results. The aim of the study was to: 1. analyze the stability of the results on the group of 20 healthy volunteers and 2. compare the MVEP measurements with the static perimetry in patients with visual field defects.

Methods 20 healthy male volunteers were examined. The procedure of the examination consisted of many measurements with the small changes of one of the parameters, i.e. time, fixation, position of the electrodes, types of electrodes, etc. 5 patients with visual field defects were examined by electrodiagnostic techniques using full-field, half-field, and quadrant field VEP, PERG, and MVEP.

Results Baseler et al. suggested that the clinical utility of the MVEP was limited because of the great variation of responses obtained from identical locations in normal individuals. At present, in many studies, the benefits of the use of MVEP, especially when combined with static visual field, are argued. Like every diagnostic technique, MVEP has its problems and limitations. They include the need of visual acuity correction, the assessment of lids position (which can occlude the vision), and the necessity of eye movements monitoring.

Conclusion Our study shows that fixation is the main factor influencing the repeated reliability of the results. The study also included different attempts at motivating the fixation of the patient (apart from the monitoring of fixation).

■ 240

Oscillatory potentials under the thyroid-associated eye's disease

SEIDOVA SFG (1), PANTELEEEVA OG (2), SHAMSHINOVA AM (1)

(1) Clinical Physiology of Vision, Moscow

(2) Oncology diseases, Moscow

Purpose To investigate oscillatory potentials (OP) for localization of the pathological process under thyroid-associated eye's disease (TAED) and to understand the mechanisms of the visual functions disorders by this pathology.

Methods 40 patients with TAED and 20 normal subjects were investigated. The age 20–50 years. OP and other types of ERG were investigated (MBN Russia). The orbit investigated by using KT and Ultrasonic. We used nonparametric statistics - median (M) and percentiles 30 and 70 (P30, P70).

Results OP had 4-5 oscillations in normal subject's, depending on age OP index was M-14.8; P30-12.2; P70-19.8. In TAED early compensation stage OP index was reduced on 30%(M-10; P30-7.7; P70-15). These datas were significantly different from normal one ($p<0.01$). They changed from normal (M-15; P30- 14; P70-17) to subnormal (M-7; P30-6.6; P70-7.7) depending on duration of the pathological process in the orbit in compensation stage. OP index changed from subnormal (M-10.7; P30- 10; P70-12) to very subnormal(M-6.5; P30- 5; P70-10) in subcompensation stage depending on duration of TAED. OP index was subnormal in decompensation stage, but if visual optic neuropathy was strongly pronounced they there unrecorded.

Conclusion The disorder of the tissue and muscles of the orbit were demonstrated with KT and ultrasonic dates. The pathology state of the retina as well as optic nerve was noted. The compression of the optic nerve and vascular network in the orbit and retina ischemia explain the mechanisms of the visual functions disorders.. Diminished OP-index reflected extent of the ischemia of inner layers of retina in all stages of TAED. OP depend on duration of the pathological process, more then clinical stage of TAED.

■ 241

Photopic negative responses to long duration stimuli in the mfERG of primary open-angle glaucoma patients

TODOROVA MG, PALMOWSKI-WOLFE AM, FLAMMER J, ORGUL S
Department of Ophthalmology, University Eye Hospital, Basel

Purpose The attempt of our study was to evaluate whether the photopic negative response (PhNR) to a long duration stimulus mfERG is sensitive to detect early retinal dysfunction in POAG.

Methods On-off mfERGs were recorded from 15 NTG and 15 HTG patients and compared to 15 controls. Recording parameters: LED stimulus screen (RetiscanTM), 212ms duration recording, stimulus matrix of 61 elements, frame rate: 70Hz, Lmax: 180cd/m², Lmin: 0cd/m², filter setting: 10-200 Hz. The PhNR following stimulus onset (PhNR-on) as well as following stimulus offset (PhNR-off) were analysed as an overall response and in quadrants as well as in 4 small central and peripheral neighbouring areas per quadrant.

Results The latency of the PhNR-on was significantly delayed in HTG in all response averages tested, while in NTG this was only seen in the small central response averages ($p<0.05$). The most sensitive measure in HTG was the latency of the PhNR-on of the small peripheral response average of the superior temporal quadrant with an area under the ROC curve of 0.924. For NTG this was the latency of the PhNR-on of the small central response average of the superior nasal quadrant with an area under the ROC curve of 0.793.

Conclusion Our results confirm that the PhNR is affected in glaucoma. In contrast to previous studies, we did not find significant differences in the amplitude of the PhNR, but in a latency delay of the PhNR-on in POAG patients. The latency delay of the PhNR-on was more sensitive for HTG than for NTG.

■ 242

Selective ERG b-wave reduction in mice deficient for anion exchanger 3

SALIVE Y (1, 2), ALVAREZ BV (2, 3), PINILLA I (4), MACDONALD IM (1), CASEY JR (2, 3)
*(1) Ophthalmology, Edmonton
 (2) Physiology, Edmonton
 (3) Biochemistry, Edmonton
 (4) Ophthalmology, Zaragoza*

Purpose Bicarbonate (HCO₃⁻) transport and metabolism are essential to ocular function. The anion exchanger 3 (AE3) catalyzes the electroneutral Cl⁻/ HCO₃⁻ exchange across the plasma membrane of retinal cells. AE3 is expressed primarily to Müller and horizontal cells. Due to its retinal location, AE3 likely contributes to the frontline defense against the tremendous CO₂ load generated from photoreceptor metabolism. To test this hypothesis, we examined retina function in mice deficient for the AE3 gene.

Methods Electoretinogram (ERG) was recorded in 3-4 months littermates (AE3+/+, wild type, WT; and AE3-/-, knockout, KO). Following overnight adaptation, mice were anesthetized with xylazine-ketamine and ERGs recorded under scotopic conditions, followed by 10 minutes light adaptation and recording of photopic ERGs.

Results Scotopic b-wave amplitudes elicited by -2.8 log cds/m² and higher luminance flashes were reduced in KO compared with WT mice; these differences reached statistical significance (ANOVA, $p<0.05$, n=12 eyes). Maximal b-wave amplitude was attained at flash intensity of 2.4 log cds/m² in both groups; however, there was a 33% reduction in amplitude in KO (344 ± 55 µV; error value as SEM) compared with WT mice (517 ± 103 µV). Intensity response series for the scotopic ERG a-wave as well as photopic b-wave were similar in both mouse groups.

Conclusion The specific reduction in scotopic b-wave amplitude is consistent with an inner retina defect occurring in Müller cells. Although no directly analogous human pathologies have been identified yet, the defect in AE3 deficient mice has similarities with snowflake vitreoretinal degeneration, both in term of functional phenotype and chromosomal location (2q36).

■ 243

Best Macular Dystrophy: Comparison of psychophysical performance with multifocal ERG responses in different stages of the disease

MAIA-LOPES S (1), SILVA E (1, 2), SILVA MF (1), REIS A (2, 1), CAMPOS S (2), CASTELO-BRANCO M (1)

(1) IBILI, Center for Ophthalmology, Faculty of Medicine, University Coimbra, Coimbra
 (2) Coimbra University Hospital, Coimbra

Purpose To compare psychophysical performance with multifocal ERG responses in different stages of Best Disease.

Methods To record mfERG we used a RETIScan system. Stimuli consisted of 61 hexagon arrays covering a visual angle of 30 deg. MfERG recordings were taken using DTL fiber electrodes. Custom psychophysical perimetric measures were also taken (parvocellular contrast sensitivity tasks using grating stimuli at 3.5 cycles per deg). Colour vision was assessed either with anomaloscope and Cambridge Colour Test.

Results N1 amplitudes were significantly lower in the patient group (Mann-Whitney tests, $p = 0.0015$) across all tested eccentricities. P1 mean amplitudes were also significantly reduced (Mann-Whitney tests, $p = 0.0001$), with preserved latency. Comparison of N1 and P1 amplitudes across eccentricity revealed a loss of the normal magnification factor, which demonstrates a predominant macular impairment. However peripheral responses were also significantly reduced. Central and peripheral impairment was also observed in our perimetric contrast sensitivity task that preferentially activates the parvocellular system.

Conclusion We have found evidence for both central and peripheral impairment in Best disease, as assessed by electrophysiological and psychophysical methods. Interestingly, parvocellular function, as measured by our psychophysical task, was heavily impaired in the periphery. Therefore this method has better sensitivity than electrophysiology.

■ 244

The effect of cataract on the Rarebit visual field

NILSSON M, ANDERSSON AM, MARTIN L
Clinical Neuroscience, Stockholm

Purpose To evaluate the effect of cataract on the Rarebit visual fields.

Methods The Rarebit Perimetry test (RP) (Frisén, 2002) relies on perception of very small (< 0.5 MAR) bright dots, presented on a dark background. The test area covers the 30 degree central visual field and the results are expressed as mean hit rate (MHR) i.e. the percentage of presented dots perceived. The RP test runs on a personal computer with a LCD screen. The test time is about 5 minutes. Ten subjects with senile cataract without any other known ocular disease were recruited from St Erik's Eye Hospital. They were examined with best corrected decimal visual acuity (VA) using the ETDRS visual acuity chart and RP before and at least six weeks after cataract surgery. All patients received an intraocular lens

Results Median VA increased from 0.32 (0.05 – 0.5) to 0.8 (0.8 – 1.25) and median MHR from 54.5 % (0 - 94%) to 88.5 % (34 - 95%) after surgery. There was a significant correlation between VA and MHR before surgery ($r = 0.67$ $p = 0.04$), but not after. In two patients with a preoperative VA of 0.5 and a postoperative VA of 1.0, no difference in MHR was seen after cataract surgery.

Conclusion This preliminary study indicates that RB results appears to be unaffected by cataract reducing the VA to 0.5 and is significantly correlated with preoperative VA at lower levels

■ 245

Published clinical studies: Acuity through the ages

MOUTRAY TN, WILLIAMS MA, JACKSON AJ
Ophthalmology, Royal Victoria Hospital, Belfast

Purpose This paper aims to: describe the methods stated to record visual acuity(VA) when used as an outcome measure in peer reviewed ophthalmology clinical studies; report any change or trend in the method used in VA recording over the last decade; compare the method of VA testing used in published studies between the United Kingdom and the United States.

Methods We reviewed the methods of recording visual acuity in a total of 160 studies published in two British and two American peer reviewed journals, compared between 1994 and 2004. The inclusion criteria was that: "Visual Acuity" or "Vision" must be stated in the title or as an outcome measure in the abstract; and the study must have included at least 10 patients.

Results The number of participants in the UK published papers ranged from 12 to 6831, whereas in those published in the USA the participants per clinical study ranged from 10 to 9980. The method used to assess VA was not specified in 33 of the UK published and 36 of the US published papers. In the results sections of the British publications the VA measurements presented were Snellen acuity (n=56), Logmar acuity (n=19), Cardiff cards (n=1) or matching method (n=1). Similarly in the US published results sections VA was recorded using Snellen acuity (n=61), Logmar acuity (n=12), or a Grating method (n=2).

Conclusion The authors recommend that to ensure comparable visual results between studies and different study populations it would be useful if clinical scientists would work to a standard VA testing protocol. This should include testing methods suitable for each target population including different ages and levels of visual acuity.

■ 246

Visual Acuity measures across the specialties

WILLIAMS MA, MOUTRAY TN, JACKSON AJ
Department of Ophthalmology, Royal Victoria Hospital, Belfast

Purpose To describe how distance visual acuity is measured & expressed in clinical studies, & to explore trends across specialties.

Methods The 2005 issues of 5 ophthalmology journals were examined & papers from those journals selected. Inclusion criteria were that at least 10 human subjects had to be involved, & "visual acuity" or "vision" had to be mentioned in the methods or results sections of the abstract. If a paper was selected, the full text of the paper was examined. The papers had to relate to one of 4 topics: cataract, refractive, age-related macular degeneration (AMD) or glaucoma.

Results 142 papers were included: 54 on cataract related topics, 53 refraction, 27 AMD and 8 glaucoma. 64 studies were performed in Europe, 53 in N. America, 20 in Asia & 5 elsewhere. The most common way in which vision was mentioned in methods sections was merely as "Best Corrected Visual Acuity" (35.9% of papers). This was true across all four specialties except the AMD group, in which 51.9% stated that an EDTRS chart was used. 7.0% did not make any mention of vision in methods, despite vision being part of the results. Where vision was mentioned in the methods 42.1% had an accompanying description or reference or both relating to how vision was measured. The commonest ways in which acuity results were presented were as Snellen acuities (45.8%), logMAR (21.1%), Snellen and logMAR (6.3%) or decimal (9.1%). This trend was true across each of the four specialty groups.

Conclusion There is a great variety of means by which vision is described in methods and results sections, both between and within studies. As distance acuity is a key outcome measure, & one that clinicians and patients can relate to, more rigour should be applied to its measurement and expression in clinical studies.

■ 247

Refractive errors in diabetic patients from DR 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 refractive errors and estimate frequency of myopia and hyperopia in diabetic patients from DR Congo.

Methods All consecutive patients with type 2 diabetes examined between April 1, 2005 and May 31, 2006 were included in this study. All patients underwent an ophthalmic examination, including noncycloplegic automated and subjective refraction. Refractive errors were determined by automated refraction and retinoscopic examination. Myopia/hyperopia were defined as spherical equivalent <-0.5 diopters/> $+0.5$ diopters. Spherical equivalent refraction was calculated as the spherical diopter plus one half of the cylindrical diopter.

Results There were 130 patients with type 2 diabetes. Refractive error measurements were available for 90 diabetic patients. Ages ranged from 37 to 81 years (mean age \pm SD, 56.4 years \pm 8.1). There were 31 (34%) female and 59 (66%) male. Mean duration of diabetes was 7.9 years and the mean blood glucose level was 160.4 mg/dl. Mean refractive errors were 0.16 diopters for the right eye and 0.92 diopter for the left eye. Frequency of refractive errors were as follows: hyperopia (48%), myopia (22%) and emmetropia (30%). Mean spherical equivalent refractions in the right eye were -2.01 ± 1.45 diopters for myopic patients and $+1.20\pm 0.48$ diopters for hyperopic patients.

Conclusion Compared with the distribution of the refraction status of general population of patients previously published in DR Congo, this study shows a high frequency of hyperopia in diabetic patients.

■ 248

Peripheral refraction in myopes and emmetropes

GONCALVES ROSADO JL, NASCIMENTO SMC
Dept of Physics, BRAGA

Purpose The aim of this study was to estimate peripheral refraction along the 45° / 225° visual fields as well as the horizontal and vertical.

Methods An infrared videoretinoscope "Plusoptix CR03" was used. The individual eccentricities of 10°, 20°, 25°, 30° were used on the nasal, temporal, superior, inferior, 45° and 225° visual fields in a myopic (n=29) and emmetropic group (n=31). A paired t - test was used.

Results Refraction was expressed as M, J0 and J45 components. For horizontal visual field, M in myopes did not change significantly with eccentricity ($P > 0.01$). For the emmetropes there was more myopia in the nasal field. J0 showed myopic shifts into the periphery in both myopic and emmetropic groups. The variation for the J45 component in the horizontal visual field was small for both groups. For the vertical field, M in emmetropes and myopes changed to a more myopic refraction and more so in the inferior part of the visual field where ($P < 0.01$). J0 had positive changes in both superior and inferior fields for both groups ($P < 0.01$). The J45 showed a positive change on the inferior part of the visual field and a negative one in the superior part in both groups. Along the 45° – 225° visual fields, the M showed a small myopic shift in the myopic group; for the emmetropes this myopic shift was greater along the 225° visual field ($P < 0.01$). J0 only showed significant changes in the 45° visual field where a positive shift for both refractive groups was observed ($P < 0.01$). The J45 presents a positive shift in both 45° and 225° visual fields.

Conclusion The data in the oblique 45° – 225° visual fields is different from that the vertical and horizontal fields, and may be useful to better quantify the eye total peripheral refraction.

■ 249

Uncorrected ametropias and visual symptoms

SORO MARTINEZ MI, VIDAL LOPEZ J, SOBRADO CALVO P,
SANCHEZ-MIGALLON MC
Ophthalmology, Murcia

Purpose The aim of this study was to evaluate the symptomatology produced by uncorrected myopia (Mp) and hyperopia (Hp).

Methods Ametropias were measured using two objective methods: autorefractometry (AR) and retinoscopy (Ret). To measure the symptomatology, a test with 26 items was used. The measurements were carried out in 156 subjects (99 boys and 57 girls). Two variables were considered: the degree of ametropia (independent variable) and the symptomatology (dependent variable).

Results The mean age of the sample was 13.32 ± 1.02 years, in a range from 12 years to 16.42 years. To obtain the uncorrected spherical ametropia, the average between the spherical equivalents obtained with the AR and with the Ret was subtracted from the spherical equivalent of the spectacles. The direct scorings obtained were transformed into percentile scores and these results were divided into five groups: high Mp, low Mp, emmetropia, low Hp and high Hp. Contrasts of averages were carried out (Student-t test) for independent variables with the scorings obtained for each item between groups. The results showed that the high Hp group was different from the other four groups in 4 variables: item 1 (eye tiredness when the subjects read during short periods of time), item 5 (they had a history of family headaches), item 15 (eyes strain when they look to nearby objects), item 26 (they feel their eyes burn). In addition, subjects with high MP and high HP get dizzier than the other groups when travelling by car (item 14).

Conclusion High uncorrected hyperopia induces more symptomatology than the rest of ranks and types of uncorrected ametropias. Dizziness when travelling by car occurs more frequently in high uncorrected ametropias.

■ 250

Evolution of the ametropias in different educational levels

JAVALOYES MORENO B, VIDAL LOPEZ J, SOBRADO CALVO P,
GONZALEZ MARTINEZ M, SANCHEZ-MIGALLON MC,
SORO MARTINEZ MI
Ophthalmology, Murcia

Purpose The aim of this study was to investigate the status of the refractive errors at different educational levels (elementary school, secondary school and university).

Methods The study was made in a sample of 703 subjects (329 girls and 374 boys) spectacles' users, distributed in three educational stages: elementary school (ESc; n=363), secondary school (SSc; n=271) and university level (UL; n=61). The spherical and cylindrical power of the right (OD) and left eye (OS) from the spectacles of each subject was registered. Two variables for each eye were considered: spherical equivalent (SE) and cylindrical power (CP).

Results For the comparison between ESc and SSc students, there was not a significant variation in the SE from the OD ($t = -0.78$, df = 608, $p = 0.39$) and the OS ($t = -0.52$, df = 607, $p = 0.60$). The same result was obtained for cylindrical power, OD ($t = -0.77$, df = 608, $p = 0.44$) and OS ($t = 0.46$, df = 607, $p = 0.65$). For the comparison between SSc and UL students, there was a significant variation in the SE from the OD ($t = 3.85$, df = 79.14, $p < 0.001$) and the OS ($t = 4.08$, df = 79.18, $p < 0.001$), showing that university student have more myopic values. Finally, there was not a significant variation in the cylindrical power neither for the OD ($t = 0.19$, df = 239, $p = 0.85$) nor for the OS ($t = 1.58$, df = 239, $p = 0.12$).

Conclusion The results obtained in this study document that the spherical refractive error tends to change from the Elementary School to the University Level, but that the cylindrical refractive error undergoes little variation.

■ 251

The effect of ocular aberrations with two different contact lenses

LINDSKOOG PETTERSSON A, JARKÖ C, BRAUTASET RL
Karolinska Institutet, Stockholm

Purpose To compare the residual spherical aberration after having fitted an aberrational controlled contact lens (ACCL) and a conventional lens.

Methods 22 healthy subjects, 20-37 years old, were recruited from the School of Optometry, Karolinska Institutet, Stockholm, Sweden. All subjects had Snellen visual acuity 1.0 or better and a refractive error between -0.50 and -8.0 D. Aberrations were measured using a wave front analyser (Zywave, Bausch & Lomb) in each subject's right eye (1) uncorrected; (2) after having worn a ACCL for 3 days (Definition AC daily disposable, Optical Connection Inc.); (3) and with the conventional lens (Dailies, Ciba Vision). The lenses were fitted with a cross over design.

Results The mean uncorrected spherical aberration was positive, 0.022 mm (± 0.0244 SD). After correction with the conventional lens the mean spherical aberration was close to zero, 0.002 mm (± 0.0227 SD). The aberration controlled lens made the mean total spherical aberration negative, -0.021 mm (± 0.0255 SD). There was no statistically significant correlation ($p > 0.05$) neither between the amount of aberration and visual acuity, nor between aberration and contrast sensitivity.

Conclusion The result indicates that an aberration controlled contact lens is only useful when the amount of spherical aberration is more than 0.1 mm.

■ 252

Ultrasonic estimating of accommodation changes after the treatment of myopic eyes

KRIAUCIUNIENE L, PALINKSNIS A
Medical university, Kaunas

Purpose The goal of our work is to evaluate the anatomic measurements of the eye using precise ultrasonic biometry for children with myopic refraction and their changes after the treatment with the low frequency electromagnetic field.

Methods 70 myopic children (140 eyes) with the accommodation spasm were estimated with the ultrasonic biometry before and after the treatment with the low frequency electromagnetic field. The age of the myopic children ranged between 6 and 15 years old and the size of the refraction was from -1.0 till -3.0 D.

Results After the treatment the clinical size of the refraction reduced but only statistic unreliable. Before the treatment the myopic children's crystalline lens when fixing the sight to the length and to the nearness didn't change. After the course of the treatment the opportunities of the accommodation of the myopic children have improved, it shows the statistic reliable changes in the eye measurements, when fixing the sight to the length and to the nearness (the difference of thickness of the crystalline lens was 0.187 ± 0.152 mm in 1st group and 0.181 ± 0.153 mm in 2nd group, in both cases $p = 0.001$).

Conclusion After the treatment children's myopic eyes by low frequency electromagnetic field during accommodation the excursions of lens crystalline thickness significantly increases ($p < 0.05$ in all cases).

■ 253

Assessment of real-time changes in wavefront aberrations with accommodation using the irx3 aberrometer

CHATEAU N (1), NGUYEN KHOA JL (2), LOPEZ GIL N (3), GICQUEL JJ (4)

(1) *Imagine Eyes, Orsay*(2) *Hôpital Foch, Suresnes*(3) *Universidad Murcia, Murcia*(4) *Centre Hospitalier Universitaire, Poitiers*

Purpose The time characteristics of accommodation may be critical in real-world situations such as car driving. Therefore a diagnostic technique is needed to evaluate the real-time performance of new accommodation recovery techniques, such as accommodating IOLs. The aim of this study was to explore the capacity of aberrometry to measure time-resolved ocular aberration changes during accommodation.

Methods We assessed ocular aberrations using a Maxwell Hartmann-Shack aberrometer (Imagine Eyes, Orsay, France) with programmable fixation target. Four healthy subjects aged between 23 and 29 were submitted to the following accommodative stimulation: (i) the target was set at the eye's far point for 5s; (ii) the target abruptly translated by 3D towards the eye and maintained this near vision position for 5s; (iii) the target rapidly moved back to the far point position and kept it for another 5s. The aberrometer software was modified in order to acquire wavefront data at a frequency of 15Hz throughout the stimulation process. Each measurement record consisted in 225 Zernike polynomial expansions up to the 8th order.

Results All subjects accommodated after a time lag of approximately 1s, with a defocus lag comprised between 1.0 and 1.5D. The return to the non accommodated state was usually faster, with a typical lag of less than 0.5s. We also observed a negative-oriented change of spherical aberration with accommodation, comprised between 0.05 and 0.2μm. The total higher-order RMS aberration was either stable, or reduced by no more than 0.1μm.

Conclusion Dynamic aberrometry provides a convenient objective technique to assess spatially-resolved and time-resolved changes in refractive error during accommodation.

■ 254

VCD/CR or AL/CR which is the best predictor for refractive changes in young adults

JORGE J (1), BORGES DE ALMEIDA J (2), PARAFITA MA (3)

(1) *Physics Optometry, Braga*(2) *Physics, Braga*(3) *Surgery Ophthalmology, Santiago de Compostela*

Purpose To compare anterior chamber depth/corneal radius ratio (ACD/CR), vitreous chamber depth/corneal radius ratio (VCD/CR) and axial length/corneal radius ratio (AL/CR) in order to establish which is the best predictor for the refractive alterations in young adults.

Methods 118 university students with a mean age (mean ± standard deviation) of 20.6± 2.3 years were enrolled in a 3-year longitudinal study. Measurements included subjective cycloplegic refraction, ocular biometry with A-scan ultrasonography, and corneal topometry by autokeratometry. Measurements were carried out at two different examinations over the period of 2002 to 2005. Binary logistic regression was used in order to quantify the effectiveness of each parameter as a predictor of refractive error alteration. As no hyperopic shifts were observed within the study population refractive change was defined as a myopic shift equal or above -0.50 D. The Wald statistic was used to establish the probability of an alteration to occur; goodness-of-fit was assessed by the Hosmer-Lemeshow method (H-L). The predictive ability of the model was also assessed.

Results For the VCD/CR the Wald statistic value was 6470.14 ($p=0.002$) and the H-L value was 0.325 with a predictive ability of 79.9%. For the ACD/CR the Wald statistic value was 6563884.26 ($p=0.021$) and the H-L value was 0.026 with a predictive ability of 77.1%. For the AL/CR the Wald statistic value was 5310.82 ($p<0.0001$) and the H-L value was 0.198 with a predictive ability of 7%. Thus, the VCD/CR ratio showed the best predictive ability and the greater H-L value within the parameters under investigation.

Conclusion The ratio VCD/CR was the best predictor of the myopic shift within a population of Caucasian young adults.

■ 255

Reliability of the measurements taken by the students of the Murcia University School of Optometry (Spain)

SOBRADO-CALVO P, JAVALOYES-MORENO B, VIDAL LOPEZ J, GONZALEZ MARTINEZ M

Ophthalmology and Optometry - University of Murcia, Murcia

Purpose The aim of this study was to investigate the reliability of several anatomical and functional measurements taken by students of Murcia University School of Optometry during their clinical internships in Contactology.

Methods In the present study the results collected by the students from the following variables were analyzed: corneal radius of curvature with keratometer ($n=217$ eyes), horizontal corneal diameter ($n=110$ eyes), horizontal pupil diameter ($n=110$ eyes), amplitude of blinking ($n=110$ eyes) and frequency of blinking ($n=105$ eyes). Measurements for each eye were taken by different examiners in different days. An analysis of reliability of the results (alpha test) with 5 measurements per variable was made using a Statistical software (SPSS v.11).

Results The measures of the corneal radius of curvature were very stable throughout the measurements ($\alpha=0.97$) showing significant stability between intersubject values ($F=2.55$, $p=0.04$). Although statistical significance was not reached, the rest of the analyzed variables showed very reliable values [corneal diameter: $\alpha=0.91$, $F=1.45$, $p=0.22$; pupil diameter: $\alpha=0.88$, $F=0.69$, $p=0.60$; amplitude of blinking: $\alpha=0.84$, $F=0.93$, $p=0.45$; frequency of blinking: $\alpha=0.89$, $F=0.74$, $p=0.56$].

Conclusion The anatomical and functional measurements taken by the students of the School of Optometry of Murcia are very trustworthy. The lack of consistency in some measurements could be due to the changing nature of the variables studied (i.e. pupil diameter or frequency of blink) or to the inadequate system to collect the data. Later investigations must be carried out to determine the reason for this.

■ 256

An Experimental Validation for a New Method of Sclera Reinforcement and Metabolic Therapy of Progressive Myopia

LAZUK AV, IOMDINA EN, ANDREEVA LD

Helmholtz Research Institute for Eye Diseases, Moscow

Purpose Development of a new method of low-invasive sclera reinforcing therapy of progressive myopia and prevention of metabolic disorders in the shells of a myopic eye, which envisages a combined effect of the basic polymer composition for the sclera strengthening injection (SSI) and an ophthalmic medicinal film (OMF) with a coordination compound of zinc and pyridoxine (the pyrazin preparation).

Methods OMFs with pyrazin, pretreated with polymeric composition used in the SSI, were located, once, under the Tenon's capsule of 24 rabbit eyes. After 2 weeks, 1, 3, 8 or 12 months the eyes were enucleated and morphologically studied.

Results The experimental studies showed a good effect of pyrazin on the processes of collagen biosynthesis, free of local irritation reaction or any other negative reaction of eye structures induced by the preparation or the polymeric carrier. The introduction under the Tenon's capsule of pyrazin-containing OMFs treated with polymeric composition for SSI stimulates growth and maturation of connective tissue on the scleral surface. Within 6-8 months, the introduced material dissolves and is fully replaced by a newly formed connective tissue tightly interwoven with the recipient sclera. Clinical testing of the technique inspired by these results has been started.

Conclusion The obtained data enable one to view the use of ophthalmic medicinal films with pyrazin pretreated with polymeric composition as a promising means of low-invasive sclera-strengthening and metabolic treatment of progressive and complicated myopia.

■ 257

The Association of Vision Science Librarians: Providing Information and Information Resources for Vision Care, Research and Education Worldwide

SIEVING P (1), SCHAEFFER-YOUNG J (2), ASSOCIATION OF VISION SCIENCE LIBRARIANS (3)

(1) *National Institutes of Health Library, Bethesda*

(2) *Wills Eye Hospital, Philadelphia*

(3) *-, Berkeley*

Purpose To introduce EVER members to The Association of Vision Science Librarians, an international group of information professionals working in ophthalmology, optometry, corporate, university, and association libraries to build collections and provide information critical to the educational, clinical, research and administrative programs of our institutions.

Methods This poster identifies the Association's services, programs and publications, and highlights our activities, with emphasis on our resource sharing and advocacy programs. Group and individual research projects are also presented.

Results Information resources are critically important for basic and clinical research, education in ophthalmology and vision-related sciences, and for the provision of the best clinical care. AVSL members work to provide these resources and related services available as efficiently and effectively as possible, and to advocate for increased access world-wide.

Conclusion AVSL members welcome contacts with new members and potential users of our Web-based resources. We hope EVER members will stop by the poster for an introduction, or visit our Web site.

■ 258

The Impact of Open Access on the Vision Literature

SIEVING P (1), ANTON B (2), ASSOCIATION OF VISION SCIENCE LIBRARIANS (3)

(1) *National Institutes of Health Library, Bethesda*

(2) *Fong Optometry Library, Berkeley*

(3) *-, Berkeley*

Purpose To provide an update on new opportunities in communication of scholarly research, with an emphasis on Open Access models of dissemination of peer-reviewed scientific literature and institutional repositories.

Methods The Open Access movement is documented; factors contributing to the impetus and need for this change in the traditional publication process are identified, along with copyright and intellectual property concerns. Several Open Access models are presented, including recent funder mandates that research results be freely disseminated. The potential impacts of these opportunities on the vision literature, including sustainability concerns for professional organizations, are discussed. Institutional repositories another new form of scholarly communication, are also presented.

Results Developments in Open Access to the vision literature over the past 10 years demonstrate both reluctance to change a system which has served the profession well, and rapid adoption of some new opportunities. "Born Open" journals are identified, as well as those titles participating in PubMed Central and other programs providing free access to journal archives. Impact factors are one measure of both acceptance and impact; recent studies on the effects of Open Access on impact factors will be presented. Participation in funder-mandated programs, such as that of the National Institutes of Health and the Wellcome Trust, is examined. Institutional repositories, which offer new opportunities, also provide a measure of change and potential impact on vision research and clinical care.

Conclusion The electronic environment and nearly universal access to the Internet offer new opportunities to increase access to information and advance science.

■ 301 / 2467

Short term effect of intravitreal Bevacizumab (Avastin) for exudative age-related macular degeneration

CHRSTMANN S (1), CORDES AK (1), DOHM S (1), SCHRAGE NF (1), DEGENRING RF (1, 2)

(1) Dept. of Ophthalmology Cologne-Merheim, Cologne Hospitals, Cologne

(2) Faculty of Clinical Medicine Mannheim, University of Heidelberg, Mannheim

Purpose To evaluate the clinical effect of intravitreal bevacizumab injections in exudative age related macular degeneration (AMD).

Methods Retrospective study. 16 eyes (16 patients) suffering from exudative AMD of all types were treated by single or repeated intravitreal injections of 1 mg Bevacizumab (Avastin). Mean number of injections was 2.3 +/- 0.9 (range, 1-3 injections; median, 3 injections) in intervals of 4 weeks. Fluorescein angiography (FA) was performed preoperatively and regularly during follow-up. Available pre- and postoperative FAs were mixed and then qualitatively compared by a masked person. Mean patient age was 73.9 +/- 8.7 years (range, 55-88 years).

Results Visual acuity (VA) increased significantly ($p=0.032$; t-test for paired samples) from preoperatively 0.16 +/- 0.36 (range, 0.03 to 0.8; median, 0.2) to 0.20 +/- 0.39 (range, 0.05 to 0.6; median, 0.3) at the end of follow-up. In 8 eyes (50%) VA improved at least one line at the end of follow up, in 5 eyes (31%) VA was stable, and in 3 eyes (19%) VA deteriorated one or two lines. In average, VA improved 1.1 +/- 2.1 lines (range, -2 lines to +5 lines; median, 0.5 lines). In 12 of 16 FAs (75%) the masked examiner judged the postoperative angiogram to be „better“, in 3 FAs (19%) the postoperative FA was graded to be „worse“, and in 1 case (6%) the examiner was „undecided“. No ocular adverse events like inflammation, retinal detachment or increase of the intraocular pressure were observed.

Conclusion In this retrospective study of single or repeated intravitreal bevacizumab injections in exudative AMD we found significant improvement of visual acuity and qualitative improvement in fluorescein angiography during short term follow-up.

■ 302 / 3266

Internal limiting membrane peeling during macular hole surgery: a comparison of outcomes with and without indocyanine green and trypan blue assistance

LAMBLEY RG, BATRA R, EWINGS P, GRAY R

Taunton and Somerset Hospital NHS Trust, Taunton

Purpose To compare anatomical and visual outcomes of macular hole surgery using indocyanine green (ICG), trypan blue (TB) or using no stain to visualise the internal limiting membrane (ILM).

Methods Retrospective analysis of 50 consecutive cases of idiopathic macular hole undergoing pars plana vitrectomy and ILM peel with or without ICG or TB assistance. ICG was used in 12 eyes, TB in 24, and the remaining 14 eyes underwent ILM peel without a stain. Where stains were used, 0.1ml of 0.5% ICG or 0.1ml of 0.15% TB was injected over the macula and left for one minute before aspiration. Outcome measures were anatomical hole closure and change in Snellen visual acuity (VA).

Results The mean number of Snellen lines of improvement in visual acuity was greater in the no-stain group (3.71 lines) than in the ICG group (2.33 lines) and the TB group (2.63 lines; $p=0.07$). 7/14 (50.0%) of the no-stain group had a final VA of 6/12 or better compared with 5/12 (41.7%) in the ICG group and 12/24 (50.0%) in the TB group. Anatomical hole closure was achieved in 10/12 eyes (83.3%) in the ICG group, 23/24 (95.8%) eyes in the TB group, and 12/14 (85.7%) eyes in the no-stain group.

Conclusion ILM peeling without a stain to help visualization was associated with higher number of lines of improvement on Snellen acuity testing than ICG and TB-assisted peeling, and with similar anatomical success. Previous clinical studies have linked poor surgical outcomes with ICG toxicity; our results indicate a similar toxic effect of TB.

■ 303 / 3267

TEM of epiretinal tissue in diabetic macular oedema

SYNEK S (1), PAC L (2), SYNKOVA M (1)

(1) Ophthalmology and Optometry, Medical Faculty, Masaryk University, Brno

(2) Anatomy, Medical Faculty, Masaryk University, Brno

Purpose Diabetic cystoid macular oedema (DME) is common cause of visual acuity decrease. Good anatomical results and visual acuity (VA) of pars plana vitrectomy (PPV) in a case of macular hole with internal limiting membrane peeling leads to usage of this technique in DME. Favourable results even in a case without vitreoretinal traction leads to conclusion that pathogenesis of this disease is different.

Methods 21 eyes from 19 patients with DME that had undergone PPV and peeling ILM were analyzed retrospectively. Half of them were laser treated before surgery. All eyes had an attached posterior hyaloid's membrane in the macular region, but without thickening and without traction. Parts of excised tissues were examined by transmissive electron microscopy (TEM).

Results Median duration of DME at the time of PPV was approximately 11.0 months. The median preoperative best-corrected VA of 0.08, improved to a median postoperative VA of 0.25. 7 eyes without preoperative laser coagulation had a median VA improvement of 77%, while 12 eyes with preoperative macular laser treatment had a median VA improvement of 14.8%. In all 21 eyes, DME was no longer visible on microscopic examination after a median period of 3.0 months after PPV. TEM samples contained ILM, glial cells and connective tissue and can be classified in monolayer membrane, multilayer membrane and true epimacular fibrous membrane.

Conclusion PPV and peeling ILM resulted in the resolution of oedema, with an improvement in visual acuity in the majority of cases. Eyes without preoperative macular photoocoagulation had a significantly higher visual improvement than eyes with preoperative laser treatment. A randomized controlled prospective trial of PPV versus laser is needed to determine the role of PPV as treatment modality for DME

■ 304

Sedoanalgesia in laser photocoagulation of retinopathy of prematurity

AKKÖYLÜN I (1), KARAASLAN P (2), YILMAZ G (1), DÖNMEZ A (2), ESEN A (1), AKOVA YA (1)

(1) Dept. of Ophthalmology, Baskent University, Faculty of Medicine, Ankara

(2) Dept. of Anesthesiology, Baskent University, Faculty of Medicine, Ankara

Purpose To report our experience with sedoanalgesia during laser photocoagulation therapy in retinopathy of prematurity (ROP).

Methods Patients files and anesthesiological records of 80 premature patients were evaluated retrospectively. Gestational age was average 29.6 ± 1.9 weeks, birthweight was average 1230 ± 265.3 grams. Out of 160 eyes, 60 were diagnosed with prethreshold and 100 with threshold ROP. All eyes underwent laser photocoagulation. Sedoanalgesia with ketamine and midazolam was performed in 80 patients. Ophthalmic complications as well as saturation, apnea, tachycardia and bradycardia were evaluated.

Results During laser photocoagulation there were no anterior or posterior segment complications. In 2 eyes, hyphema developed 1 day after laser photocoagulation. All eyes showed regression of ROP after laser photocoagulation. During sedoanalgesia apnea was registered in 10.4%, desaturation in 4%, tachycardia in 2% and bradycardia in 2% of the patients. None of the patients needed intubation.

Conclusion Sedoanalgesia could be an option during laser photocoagulation of ROP in order to prevent complications of general anesthesia.

■ 305

Biennial Screening for Mild Nonproliferative Diabetic Retinopathy

GORDON-BENNETT PSC (1), NEWSOM W (1), FLANAGAN DW (1, 2, 3)

(1) *Ophthalmology, Hinchingbrooke Hospital, Huntingdon*(2) *Ophthalmology, Addenbrookes Hospital, Cambridge*(3) *Ophthalmology, Moorfields Eye Hospital, London*

Purpose To determine whether diabetics being screened annually for diabetic retinopathy (DR) could be safely screened biennially and to identify low-risk characteristics.

Methods 658 diabetics were screened for DR. Their retinal screening photographs 1 year, 2 years and 5 years prior were examined. The duration of diabetes, blood pressure, HbA1c and cholesterol were noted.

Results Of 160 mild DR, 1 year before, 64 had mild DR, 54 had no DR; 2 years before, 42 had mild DR, 48 no DR; 5 years prior, 12 had mild DR and 40 no DR. Of 20 moderate and severe DR, there were 9 mild DR and 3 mod DR at 1 year. Out of 250 controls, 5 had previous mild DR, 98 had no previous photos and 147 and no previous DR.

Conclusion Mild DR can be safely screened at 2 year intervals provided HbA1c, BP and cholesterol are controlled.

■ 306

Improving attendance at the diabetic retinopathy screening at East London

PETO T (1), SCHMERMER C (1), POWLING A (2), DIABETIC RETINOPATHY SCREENING TEAM THE (1, 2)

(1) *Moorfields Eye Hospital, London*(2) *Tower Hamlets Primary Care Trust, London*

Purpose To improve attendance at the East London Diabetic Retinopathy Screening (DRS) Program

Methods A team, comprising of a clinician, screeners and administrative personnel was formed to improve Did Not Attend (DNA) rate. First, the accuracy of the database was determined by cross-examining relevant hospital databases. An educational campaign about the purpose of DRS was initiated and aimed at physicians, diabetologists and religious leaders of the community. Then, Bengali-speaking clerks were employed to book appointments. Then, hospital databases were scrutinised again for attendance and those followed-up by the eye-unit were re-classified as slit-lamp screening. Employing Bengali-speaking screeners minimised the anxiety associated with screening. A second clinic in a geographically more convenient location was set up to facilitate take-up of services.

Results The estimated prevalence of diabetes in the region is 6.1%. Of these, 72% were attended screening with many more appointments sent. The database was found to be over 90% accurate. The educational campaign reached all GPs, diabetologists and religious leaders in the region. On days Bengali-speaking administrator made the bookings, DNA rate dropped from nearly 50% to 25%; for those who have already attended DRS once, it was below 10%. On hospital databases a further 202 patients were located as participating in slit-lamp screening. Bengali speaking screener at the second location brought the DNA rate down to 30%.

Conclusion The team approach to raising attendance at the DRS Program was effective as it raised the percentage of patients to 72% that is well-within the current guidelines for screening in the UK.

■ 307

Retinal vessel signs and early retinopathy in type 1 diabetes

KYTÖ JP (1, 2), ROSENGÅRD-BÄRLUND M (2), FORSBLOM C (2)

HIETALA K (2, 1), FAGERUDD J (2), LINDSTRÖM-KARJALAINEN M (1),

GROOP P-H (2), SLIMMANEN PA (1)

(1) *Ophthalmology, Helsinki University Central Hospital, Helsinki*(2) *Diabetes Genetics, FinnDiane Study Group, Folkhälsan, Helsinki*

Purpose To design a method to grade arteriosclerotic retinal vessel signs in diabetes and to evaluate their association with early retinopathy (RP) in type 1 diabetes.

Methods The preliminary cross-sectional substudy of FinnDiane comprised of 20 consecutive patients (11 men) aged 25 (SD 6), with T1D duration of 8.8 (1.4) yrs. One macula and one optic disc centered 50–60° red-free fundus image were snapped and digitized. From patients with RP (n=10) the more affected eye and from patients without RP a random eye were graded in masked fashion for 1) arteriolar (sinusoidal elongation, straightening, pronounced light streak, generalized and focal narrowing), 2) venous (beading, local narrowing and tortuosity), 3) arteriovenous nickings (AVN: remoteness, arching, banking, compression and deviation) signs and 4) RP. AVN signs were summed as AVN risk sum (ANR) and arteriolar, venous and nicking signs as arteriosclerosis risk burden (ARB).

Results Patients with RP were on average 1.1 yr younger and diabetes had lasted 1.1 yr longer. They had higher mean arterial pressure (95 vs. 91.5mmHg) and lower HDL (1.63 vs. 1.87mmol/l) but total cholesterol and HbA1c 8.2 (1.4)% were similar. Arteriolar (sinusoidal elongation n=18, generalized n=16 and focal narrowing n=1, pronounced light streak n=10, straightening n=1) and venous (tortuosity n=20, beading n=2 and local narrowing n=1) signs were common in 20 eyes. At least one AVN occurred in 19 eyes. ANR and ARB were higher in eyes with RP than without 11.7 (7.8, range 1.3-24.3) vs. 7.7 (5.2, range 0-15.2) and 36.8 (11.7, range 24.0-61.3) vs. 27.8 (12.7, range 15.7-57.1), respectively.

Conclusion Early AVN and other vessel signs seems to associate with retinopathy.

■ 308

Diabetic retinopathy progression in patients submitted to pancreatic and renal transplant

MIGUEL L (1), DORES J (2), MEIRELES A (1)

(1) *Ophthalmology, Porto*(2) *Endocrinology, Porto*

Purpose Simultaneous pancreas and kidney transplant is an effective therapeutic option in diabetic patients with end stage renal disease. The aim of this study is to assess transplant impact in the natural course of diabetic retinopathy

Methods 44 patients submitted to simultaneous pancreas and kidney transplant between May 2000 and December 2005 at Hospital Geral Santo António, Oporto, were reviewed. Each visit consisted in visual acuity assessment and routine slit-lamp and fundoscopic examinations. A thorough review of clinical data was made, namely pretransplant visual acuity and diabetic retinopathy grading. Pretransplant fotoocoagulation treatment and pretransplant vitreoretinal surgery assessment was made too

Results Full and updated data is to be presented at the congress.

Conclusion This procedure, when successful, provides an adequate metabolic control and increased quality of life. Conclusions concerning the improvement of diabetic retinopathy's natural course after transplant will be presented at the congress.

■ 309

Photodynamic Therapy (PDT) and Periocular Triamcinolone for Retinal Angiomatous Proliferation

HIGGINS GT, SCARMOULTSOS F, RILEY A, HARDING S
St Paul's Eye Unit, Liverpool

Purpose Retinal angiomatous proliferation (RAP) is a subtype of exudative age-related macular degeneration, in which neovascularization (NV) begins in the deep retina, extends through the subretinal space, and eventually communicates with choroidal NV. PDT, combined with periocular triamcinolone (TA) may be effective in treatment of RAP.

Methods 16 eyes of 14 patients diagnosed with RAP on the basis of fluorescein (FA), indocyanine green (ICG) angiography and video angiography were treated with PDT and in some cases TA. They had 3 monthly assessment and PDT treatment was repeated if necessary. Outcome data included best-corrected LogMAR visual acuity (BCVA), contrast sensitivity (Pelli Robson), FA, ICG, and presence of subretinal fluid (SRF), cystoid macular oedema (CMO), fibrosis and haemorrhage.

Results Mean age was 81.4 yrs (range 64-96), mean follow up was 17.7 mo. (range 2-35). The mean number of PDT treatments was 2.5 (range 1-5). 5 eyes also had TA with the first PDT. The frequency of stable (<15 logMAR letters lost)/improved BCVA was: 6 mo. 7/10 (70%); 12 mo. 3/6 (50%) and 24 mo. 2/4 (50%). Angiographic occlusion of RAP was seen in 3/10 (30%) at 6 mo., 3/6 (50%) at 12 mo. and 4/4 (100%) at 24 mo. There was limited follow up of PDT/TA patients (n=2 for >6/12) but at 6 months one had gained 11 letters and the other had lost only 1 letter.

Conclusion PDT in RAP did not show additional benefit over that previously reported at 12 mo. (50% significant visual loss vs. 55% for occult placebo in VIP study), though at 24 mo. there was a trend showing benefit for PDT with less frequent visual loss (50% vs. 68% for VIP study). There may be a favourable trend for adjunctive TA but there is as yet limited follow up.

■ 310

Stabilization of visual acuity after intravitreous pegaptanib injections in patients with neovascular AMD

BREUER B, JOCHMANN C, WIEDEMANN P
Department of Ophthalmology, University of Leipzig, Leipzig

Purpose Subfoveal neovascularization is the most frequent cause of severe visual loss in patients with age-related macular degeneration (AMD). Pharmacologic inhibition of vascular endothelial growth factor (VEGF) is a new principle of treatment of this condition. Pegaptanib (Macugen), an anti-VEGF aptamer, can selectively bind and inhibit VEGF(165).

Methods 11 patients with recent visual loss were included, 9 with occult and 2 with minimal classic choroidal neovascularization. 5 of 11 patients had a detachment of the pigment epithelium. Intravitreous injections of 0.3 mg pegaptanib were administered three times in 6 weeks intervals. Visual acuity was measured by ETDRS acuity charts 12-24 weeks before the first injection, on each day of injection and 6 weeks after the third injection.

Results Mean visual acuity dropped from 0.57 ± 0.25 12-24 weeks before the first administration to 0.4 ± 0.14 on the day of the first administration ($p < 0.05$). At our study end point 24 weeks after the first administration visual acuity was 0.36 ± 0.16 . From the first pegaptanib injection to the end point of our study no statistically significant drop of the mean visual acuity could be detected. Intravitreous pegaptanib injections were well tolerated without any adverse events.

Conclusion Intravitreous injection of pegaptanib was well tolerated and appears to be safe and effective in the stabilization of visual acuity in patients with neovascular AMD.

■ 311

Impairment of the ubiquitin-proteasome pathway by 7-ketcholesterol on ARPE-19 cells: contributions to AMD

OLIVEIRA FERREIRA JV (1, 2), GIRÃO H (2), PEREIRA P (2)
(1) Department of Zoology of the Science and Technology Faculty of the University of Coimbra, Coimbra
(2) Center of Ophthalmology of IBILI- Biomedical Institute of Investigation in Light and Image, Coimbra

Purpose Oxidized low-density lipoproteins (oxLDL) and products of cholesterol oxidation like 7-ketcholesterol (7K) have the ability to impair the retinal-pigmented epithelium (RPE). Our aim is to access the effect of 7K in RPE, particularly on the impairment of the ubiquitin-proteasome pathway (UPP).

Methods Cultures of ARPE-19 cells where incubated with 7K 20 µg/ml for 6 to 36 hours. Protein modifications induced by 7K were accessed by the determination of the carbonyl groups. Protein-ubiquitin conjugates were determined by western blot. The effect of 7K on proteasome activity was determined using a fluorogenic substrate.

Results The results presented in this study show that incubation of RPE cells with 7K leads to a time and concentration dependent increase in the levels of protein-ubiquitin conjugates. The conjugates increase is not related to proteasome inhibition (as evaluated by the ability to degrade a fluorogenic substrate) but rather to an increased availability of substrates created by 7K induced accumulation of protein-carbonyls.

Conclusion Accumulation of 7K in RPE during aging contributes to an increase in polyubiquitinated proteins, due to 7K induced protein modification, leading to RPE dysfunction associated with AMD.

■ 312

Autologous choroid-RPE-sheet translocation in exudative AMD

DEGENRING RF (1, 2), CORDES AK (1), DOHM S (1), SCHRAGE NF (1)
(1) Dept. of Ophthalmology Cologne-Merheim, Cologne Hospitals, Germany, Cologne
(2) Faculty for Clinical Medicine Mannheim, University of Heidelberg, Mannheim

Purpose To evaluate the clinical results after autologous choroid-RPE-sheet-translocation in exudative AMD.

Methods The retrospective study included 8 eyes (7 patients), that were affected by progressive visual acuity (VA) loss due to choroidal neovascularisation (CNV) due to exudative AMD. As a ultima ratio all eyes were treated by standard pars plana vitrectomy, 180-degree-retinotomy, extraction of the CNV, translocation of a choroid-RPE-sheet from the periphery to a subfoveal location, silicone oil endotamponade, and cataract surgery in phakic eyes. VA preoperatively was hand movements (HM) to 0.2 (median 1/30). Follow-up was 21 ± 16 Weeks (median, 20 weeks).

Results At the end of follow-up VA was HM to 0.3 (median, 1/30). VA improved in 5 eyes, was stable in 1 eye and deteriorated in 2 eyes. One eye had reading ability. In 3 eyes, who had better VA before, retinal detachments (2 rhegmatogenous, 1 PVR) lead to re-operations.

Conclusion These preliminary results suggest that choroid-RPE-sheet translocation may improve VA in selected eyes. Retinal detachments influenced the results adversely in 3 of 8 eyes. Further studies are warranted.

■ 313

The leucine (7)-to-proline (7) polymorphism in the signal peptide of neuropeptide Y is not a risk factor for exudative age-related macular degeneration

KAARNIRANTA K (1), HOLOPAINEN J (2), KARVONEN M (3), KOULU M (4),
KALLIO J (4), PESONEN U (4), TERÄSVIRTA M (1), UIUSITALO H (1),
IMMONEN I (2)

(1) Depth of Ophthalmology, Kuopio

(2) Depth of Ophthalmology, Helsinki

(3) Depth of Neurology, Turku

(4) Depth of Pharmacology and Clinical Pharmacology, Turku

Purpose Due to regulatory role of NPY in angiogenesis we wanted to analyze the association of Leu7Pro polymorphism in exudative AMD patients.

Methods The genotype analysis for polymorphism of leucine 7 to proline 7 (Leu7Pro) in the signal peptide region of the human preproneuropeptide Y (prepro-NPY) was performed from blood samples of exudative AMD patients (n=240) and control subjects (n=79).

Results Exudative AMD patients demonstrated NPY signal peptide polymorphism in 11%, while 14% of control patients carried the Leu7Pro polymorphism. There were no statistically significant differences in the Leu7Pro polymorphism frequency between the exudative AMD and control cases analyzed by Fisher's exact two-sided test.

Conclusion Polymorphism of leucine 7 to proline 7 (Leu7Pro) in the signal peptide region of the human preproneuropeptide Y (prepro-NPY) is not a risk in exudative AMD.

■ 315

Transpupillary thermotherapy (TTT) for the treatment of idiopathic choroidal neovascular membranes: A prospective study in the South Indian population

RAJA V (1, 2), CHANDRAMOHAN K (3), SHUKLA D (3), MANOJ S (3), KIM R (3),
KOSHAL RAM B (3), NAMPERUMALSAMY P (3)

(1) The University of Liverpool, Liverpool

(2) The Eye Care Centre, Leighton Hospital, Crewe

(3) Aravind Eye Hospital, Madurai

Purpose To assess the efficacy of TTT for the treatment of idiopathic subfoveal CNV in the South Indian population.

Methods In a prospective, non-randomized and interventional case study, 43 eyes with subfoveal CNV were studied. All the CNV were treated with 810 nm diode laser (TTT) using a spot size ranging from 0.8 to 3.0mm and power ranging from 180-800 mw after a test spot outside the vascular arcades. The main outcomes assessed were Snellen's visual acuity and angiographic leakage of the CNV post treatment.

Results 24 eyes with classic CNV and 19 eyes with occult CNV of the idiopathic type were treated and followed up for a mean period of 6.1 months. The mean change in vision in the classic group was -0.31 logMAR(SD 0.453) and -0.13 logMAR(SD 0.26) in the occult group. Classic membranes were closed in 58.33 % of the patients while occult membranes were closed in 63.10% of the patients.

Conclusion TTT is a potential treatment option for the management of subfoveal idiopathic subtype of CNV as it has been successful in closing the CNV while maintaining the vision in a majority of the eyes. However, other treatment options like PDT, as demonstrated by Chen et al and Spaide et al, have shown more promise in the management of this subtype of CNV. TTT seems to be of great benefit in developing countries like India since treatment options like PDT are beyond the reach of the majority of the population. Further randomized studies are needed to ascertain the efficacy of this treatment modality.

■ 314

Associated Factors for Age-Related Maculopathy in the Adult Population in China. The Beijing Eye Study

JONAS JB (1), XU L (2), LI Y (2), ZHENG Y (2)

(1) Department of Ophthalmology, Faculty of Clinical Medicine Mannheim, University of Heidelberg, Mannheim, Germany, Mannheim

(2) Beijing Institute of Ophthalmology, Beijing Tongren Hospital and Capital University of Medical Science, Beijing, China, Beijing

Purpose To evaluate factors associated with the prevalence of age-related maculopathy (ARM) in the adult Chinese population.

Methods The Beijing Eye Study, a population-based prevalence study, included 4439 subjects out of 5324 subjects from a Greater Beijing, aged 40+ years and invited to participate (response rate 83.4%). Fundus photographs were graded using the Wisconsin Age-Related Maculopathy Grading system. The following parameters were graded: drusen size, drusen type, and the area covered by drusen; pigmentary abnormalities; geographic atrophy; and exudative AMD.

Results Fundus photographs were available for 8655 eyes of 4376 (98.6%) subjects. Early ARM, late ARM, and exudative ARM, respectively, were present in 1.4%, 0.20% and 0.10% of the subjects. In a binary logistic regression analysis, early ARM was statistically associated with age ($P<0.001$; 95%CI: 1.04, 1.08), hyperopic refractive error ($P=0.008$; 95%CI: 1.04, 1.28), rural region ($P<0.001$; 95%CI: 0.17, 0.49), and lower level of education ($P=0.01$; 95%CI: 1.07, 1.65). Early ARM was not significantly associated with the optic disc size ($P=0.42$), and size of beta zone of peripapillary atrophy ($P=0.28$), the self-reported diagnosis of diabetes mellitus ($P=0.39$; OR: 1.37; 95%CI: 0.66, 2.85), amount of cortical cataract ($P=0.72$), subcapsular cataract ($P=0.98$) and nuclear cataract ($P=0.26$), gender ($P=0.23$), cataract surgery ($P=1.0$; OR: 0.96; 95%CI: 0.13, 6.95), glaucomatous optic nerve damage ($P=0.77$; OR: 0.62; 95%CI: 0.15, 2.52), and history of smoking ($P=0.66$; OR: 1.14; 95%CI: 0.65, 2.00).

Conclusion Hyperopic refractive error besides age was the single most important risk factor for ARM in adult Chinese. Other associated factors were rural region and lower level of education.

■ 316

The association between exposure to blue light and the incidence of age-related maculopathy: an epidemiologic review

SMITH AF (1, 2), MINASSIAN DC (3)

(1) Health Economics Unit, Alcon Laboratories Ltd, Hemel Hempstead

(2) Nuffield Laboratory of Ophthalmology, University of Oxford, Oxford

(3) Epidemiology Department, Institute of Ophthalmology, UCL, London

Purpose To assess the epidemiologic evidence concerning the association between exposure to visible light (sunlight and ambient light 400-700 nm) in particular blue light (400-520 nm) and shorter wavelengths of UV sunlight and the incidence (or risk) of age-related maculopathy (ARMD).

Methods A systematic search of published material was conducted using the following search engines: BioMed Central, Blackwell-Synergy, BMJ Journals, Ingenta Select, Ovid Online, PubMed, Science Direct. The search was limited to the publication years 1985 to 2004 and followed established methods of study assessment with due regard to sources of bias and justification of the conclusions.

Results Studies reporting on UV sunlight exposure and maculopathy show no association with any form of ARMD, while studies of sunlight exposure showed only a weak or borderline association with late forms of ARMD (OR=2.1 to 2.3).

Conclusion The balance of data does not provide convincing evidence that higher exposure to solar radiation in real life increases the risk of ARMD. The excess risks reported from well-designed major American studies are small and could be explained by uncontrolled confounding or other sources of bias. Nevertheless, the findings cannot be dismissed and remain suggestive of a positive association. If exposure to sunlight was a strong (clinically important) causal risk factor for ARMD, the powerful case-control and cross-sectional studies reported in Australia and Southern France would be expected to detect the relation, however, they did not do so, in spite of the potential for individuals to be exposed to high levels of solar radiation. The exposure measures reported do not comment on the duration or dosage of exposure.

■ 317

Intraocular Pressure Following Intravitreal Injection of Triamcinolone Acetonide

ANSARIEA (1, 2), ALIN (1)

(1) Eye Department, Eye Ear and Mouth Unit, Maidstone

(2) Department of Physical Sciences, University of Kent, Canterbury

Purpose To investigate the intraocular pressure (IOP) response following intravitreal injection of triamcinolone acetonide.

Methods This retrospective consecutive case series study included 41 patients (52 eyes) (19 male, 22 female; mean age 64.1 ± 13.44 ; range 22 – 85 years) with progressive exudative ARMD (n = 10 eyes) or diffuse diabetic macular oedema (42 eyes), who received one or more intravitreal injection(s) of 4 mg triamcinolone acetonide.

Results IOP increased significantly ($p<0.001$) from 16.08 (± 3.28) mm Hg (range 12–26 mm Hg) preoperatively to a mean maximum of 26.1 (± 11.79) mmHg (range 15–80 mm Hg) postoperatively ($p<0.001$). An IOP rise to values higher than 21 mm Hg was observed in 26 (50%) eyes. Elevation of IOP rise occurred 7.5 weeks (± 7.07) after the injection. In all but one eye, IOP could be lowered to the normal range with topical medication, without development of glaucomatous optic nerve head changes. In the eye with an elevation of IOP to 80mmHg, an A/C tap was performed resulting in an effective reduction in pressure to 21 and then to a final IOP of 12. All five patients (11.9%) with a family history of glaucoma developed an IOP rise above the mean maximum level.

Conclusion After intravitreal injections of 4 mg of triamcinolone acetonide, an IOP elevation greater than 21 mmHg developed in 53.8% of eyes, starting on average 7.5 weeks after the injection. In the vast majority, IOP was normalised by topical medication alone 6 months after the injection. A family history of glaucoma may predispose patients to a greater than average IOP rise following IVTA.

■ 318

Quality Assurance in the UK Macular Degeneration Reading Centre Network

MULDREW KA (1), PATTON WP (1), HARDING S (2), PETO T (3), REEVES B (4), CHAKRAVARTHY U (1)

(1) Ophthalmology and Vision Science, QUB, Belfast

(2) St Paul's Eye Research Centre, Royal Liverpool University Hospital, Liverpool

(3) Reading Centre, Moorfields Eye Hospital, London

(4) HSRU, London School of Hygiene and Tropical Medicine, London

Purpose To report on quality assurance (QA) in the newly established Reading Centre (RC) network. This network consisting of three RCs is responsible for monitoring and evaluating angiographic outcomes in all patients undergoing photodynamic therapy within the national health service of the United Kingdom.

Methods The Central Angiographic Resource Facility (CARF) receives angiograms from a network of 50 nominated treating centres and distributes them to one of three RC located in Belfast, Liverpool and London. The QA process which is integrated into the grading routine automatically selects 1 in every 8 angiograms to be re-graded in a masked fashion permitting the assessment of within grader, between grader and between site reproducibility.

Results To date, 208 quality assurance gradings have been performed. For lesion categorisation subtype 191 complete sets of regrading from QA were available. Within grader concordance was 66%. Within site concordance was 63%, 58% and 59% in Belfast, Liverpool and London respectively (overall concordance 61%). Inter-site concordance was 65%.

Conclusion Previous studies have shown considerable discordance both within observers and between observers. The present study has shown consistent within-grader concordance and good intra and inter site concordance. The results confirm that a multi-centre approach to angiographic grading is feasible and that quality assurance data from intersite gradings is comparable to intrasite findings.

■ 319

Photodynamic therapy combined with intravitreal triamcinolone in exudative AMD, carrying out early retreatment: comparative study

HERNANDEZ VERDEJO JL (1, 2), ALVAREZ MT (1), FERRER B (1), MARINA C (1), TEUS MA (1, 3, 4)

(1) Vissum Corporación Ofthalmológica De Madrid, Madrid

(2) Escuela Universitaria De Optica (UCM), Madrid

(3) Hospital Príncipe De Asturias, Alcalá De Henares

(4) Universiad De Alcalá, Alcalá De Henares

Purpose To evaluate the outcomes of combined photodynamic therapy (PDT) with verteporfin and intravitreal triamcinolone acetonide(IVTA) in the treatment by age-related macular degeneration, carrying out early PDT retreatment .

Methods 26 eyes of 26 patients were prospective recruited and treated using standard PDT with (study group) or without (control group) 4 mg of IVTA. In the study group (SG), IVTA was performed immediately after PDT. The mean number of treatments, mean logMAR best corrected visual acuity (BCVA) and the major diameter an area of the CNV at 1 year were compared between SG and control group (CG).

Results 13 patients were included in the SG and 13 in CG. In the SG the decrease in great linear dimension and total area of the CNV were statistically significant ($p=0.001$ and $p=0.003$, respectively). The mean number of treatments was 2.7 ± 1.4 in the SG and 4.1 ± 1.6 in the CG ($p=0.04$). We did not find a significant change in BCVA.

Conclusion The combined treatment with PDT and IVTA with early retreatments appeared more effective statistically at 12 months for reduction of great linear dimension and total area of the subfoveal CNV in AMD, compared with PDT monotherapy.

■ 320

Calculation of the Magnification in Optical Coherence Tomography (STRATUS OCT 3000) to measure Optic Nerve Head (ONH) size

PABLO LE (1, 2), BARAIBAR B (1, 2), SANCHEZ-CANO A (1, 2), EGEA MC (1, 2), GÜERRINI N (1, 2)

(1) Hospital Universitario Miguel servet, Zaragoza

(2) Instituto Aragonés de Ciencias de la Salud, Zaragoza

Purpose To evaluate the magnification characteristics of an Optical Coherence Tomograph (STRATUS OCT 3000) for the measurement of the optic disc size.

Methods An eye model was designed, built and used to measure the changes in the magnification of the Optical Coherence Tomograph with variation in the axial length of the eye and OCT correction lenses. Theoretical calculations were made with ray layout to compare the experimental and theoretical results.

Results For an eye of 18 mm of axial length OCT magnification can be calculated from this function: Magnificatio OCT = $-0.003 \times \text{OCTCORRECTION}(D) + 1.676$ ($r^2=0.840$), for an eye of 21 mm: MagnificationOCT = $-0.006 \times \text{OCTCORRECTION}(D) + 1.307$ ($r^2=0.929$) and for one of 24mm: MagnificationOCT = $-0.007 \times \text{OCTCORRECTION}(D) + 1.141$ ($r^2=0.859$). Increment of +3mm in axial length varies rim size in 11.8% and decrease of -3mm in 28.1%.

Conclusion Our results confirm that any variation in the optical system of the eye, in the OCT and/or in the distance between them induces a modification in the Tomograph images. These variations can affect the absolute size measurements of fundus structures of the eye.

■ 321

Foveal thickness after phacoemulsification.**A one year optical coherence tomography evaluation**

BARTESELLI G, MAESTRONI L, MIGLIOR S

Clinica Oculistica, Policlinico di Monza, Università Milano Bicocca, Monza

Purpose To evaluate macular thickness changes after uneventful cataract surgery using optical coherence tomography (OCT) and to assess correlation between retinal thickness and visual acuity.

Methods Twenty-three eyes of 19 patients (mean age 70.3 yrs) underwent uncomplicated phacoemulsification with in the bag implantation of a foldable intraocular lens. Patients with retinal diseases were not included. Best corrected visual acuity (VA) and OCT examinations were performed preoperatively and 10 days, 3 months and 12 months postoperatively. Postoperative changes in retinal thickness greater than 5% from preoperative values were considered as being statistically significant. This threshold greatly exceeded the 95% CI of coefficient of variation of 5 repeated foveal measurements attained on 10 normal volunteers (0.008).

Results VA was greater than 8/10 in 70% of the cases at the first visit, in 83% at the second visit, and in 94% after 1 year. Localized increases in retinal thickness reached a maximum at 3 months in 11 of 23 eyes (48%). The edema was primarily located in the central macular region: foveal thickness increased by 16.6% in 5 of 23 eyes at the first visit, and by 29.7% in 4 of 23 eyes at the second visit; it was still increased by 9.4% in 5 eyes after 12 months. A significant correlation between foveal thickness and visual acuity was observed only at the first visit ($r^2 = 0.28$, $p = 0.009$).

Conclusion Clinical and subclinical retinal thickness changes can be detected after phacoemulsification by using OCT. Macular edema occurred primarily in the foveal region, and about 30% of the variability of post-op VA was explained by foveal elevation. However, these changes tended to fade away after 12 months from surgery.

■ 322

Optical Coherence Tomography (OCT) In Serous Maculopathy Associated With Optic Disk Pit Managed With Vitrectomy-Laser-Gas. Case Report

PENDINO V (1, 2), SPINELLI A (2), PARISE S (1), VARANO L (1)

CARNOVALE SCALZO G (1), SCORCIA GB (1), SCORCIA V (1), SCORCIA G (1)

(1) Eye Clinic, Magna Graecia University, Catanzaro

(2) Biomeeting Day Surgery, Reggio Calabria

Purpose To report OCT-3 is useful in the diagnosis and follow-up of the serous retinal detachment associated with optic disk pit successfully treated with pars plana vitrectomy, endophotocoagulation and gas tamponade.

Methods We describe a rare case of a congenital optic disk pit of a 24-years old man who presented a serous macular detachment as first sign of a congenital optic disk pit. The patient was managed with pars plana vitrectomy associated with endophotocoagulation of the temporal edge of the disc, gas tamponade and face down positioning. Optical Coherence Tomography (OCT) was performed before and after the surgical treatment. Preoperative OCT-3 showed that the retinal elevation consisted of partial both separation of the inner and outer retinal layers and detachment of the below retinal pigment epithelium. After vitreous surgery, endophotocoagulation and intraocular gas tamponade, OCT highlighted the closure of the communication between optic nerve head pit and subretinal space, helped in monitoring reabsorption of the macular detachment and showed that improvement in central vision corresponded with flattening of the outer layer detachment in the fovea.

Results Good anatomic and functional results were obtained by surgical management with Vitrectomy-Laser-Gas demonstrated by OCT-3 after one year of follow up.

Conclusion OCT-3 is a useful tool for monitoring the therapeutic effect of vitrectomy, photocoagulation and gas tamponade for retinal detachment associated with optic nerve head pit.

■ 323

X-linked juvenile retinoschisis - age related macular changes by optical coherence tomography

LESCHB (1), SZABO V (1), PAMER Z (2), KNEZY K (1), SALACZ G (1), PAPP A (1), SOMFAI GM (1), VARSANYI B (1), VAMOS R (1), HARGITAI J (1), FARKAS A (1)

(1) Semmelweis University, Department of Ophthalmology, Budapest

(2) University of Pécs, Department of Ophthalmology, Pécs

Purpose To examine the age related macular changes optical coherence tomography (OCT) in 13 independent Hungarian families with genetically confirmed X-linked juvenile retinoschisis (XLRs).

Methods 20 affected males (mean age \pm SD: 24.5 years \pm 16.8 years) and 13 female carriers (mean age \pm SD: 37.7 years \pm 13.3 years) were involved into the following examinations: refractometry, best corrected visual acuity (BCVA) using LogMAR, biomicroscopy, and OCT3 (Humphrey-Zeiss, USA). Patients were divided into two groups by their age. 1st: age < 30 years (14 patients), 2nd: age > 30 years (6 patients). OCT scans were taken across the fovea, using internal fixation target. Foveal thickness (FT), total macular volume (TMV) of 6mm and central macular volume (CMV) of 3.45mm long scans were measured. Correlations were calculated between BCVA, age and OCT parameters.

Results The mean FT was significantly elevated in the 1st group and mildly in the 2nd group (calculated by the software), while the central FT (measured by caliper) was significantly decreased in the 2nd group. CMV and TMV were significantly ($P < 0.05$) elevated in the 1st group and significant decreased in the 2nd group. The BCVA (LogMAR) showed significant impairment in patients (1st group < 2nd group). Inverse correlation ($r^2: 0.2-0.29$; $P=0$) was observed between age and OCT parameters. Carriers data were within the age-matched normal range.

Conclusion OCT revealed cystic thickening of the macula in the young age (group 1) and atrophic changes in the older age (group 2), with diminishing of BCVA. In spite of the centrally localized disturbance of the retina visible by funduscopy, OCT indicated retinal damage beyond the macula.

■ 324

Profile of Retinal Detachment Management at Public Hospitals of National Health Service (NHS)

SANCHEZ CHICHARRO D (1), PASTOR JIMENO JC (2), ROJAS J (2)

FERNANDEZ I (2), GOMEZ-ULLA F (3), PINERO A (4)

(1) Ophthalmology, Hospital Clínico Universitario, Valladolid

(2) Ophthalmology, IOBA, Valladolid

(3) University of Santiago de Compostela, Santiago de Compostela

(4) University of Sevilla, Sevilla

Purpose To determine the profile of vitreo retinal surgery management and the after hours retinal detachment(RD) care at the hospitals of NHS in Spain

Methods 217 heads of Ophthalmology services of the NHS hospitals across Spain were asked to complete a confidential questionnaire with 30 items. Qualitative variables were analyzed by Chi-squared and Fisher's exact tests and quantitative by Kruskall-Wallis test

Results 119 answers (answer rate: 54.84%) were received. Feedback came mainly from high level teaching hospitals (TH: 67 out of 148). District general hospitals and non teaching hospitals (NTH) were grouped together (49 out of 69). Approximately 64% (75/116) of the centres perform pars plana vitrectomy (PPV) and 83% (95/115) scleral surgery. Almost 70% (48 / 69) of the performing PPV centres do over 100 programmed PPV per year and 15% less than 50 PPV. There is a mean of 2 vitreo-retina specialists (VR) per centre, the 23% of the mean staff (14 in TH and 5 in NTH). VR specialists perform a median of 50 PPV per year (60 in TH and 24 in NTH). Approximately 77% (52/68) of the centres with PPV cover urgent RD but only 36% have VR specialist on call. Furthermore 80% (95) of centres admit to have problems in handling urgent RD after-hours; due to theatre availability 24%, ophthalmic trained nurses 78%, and anaesthetists 49%. Only 40% (29/72) of centres internally audit their results (38% TH and 2% NTH)

Conclusion Despite the cautions that should be taken with sample selection and the ignorance of the non-response bias, we learned the number of programmed PPV done in the last year, problems to manage urgent RD, specialists involved in vitreoretinal diseases care, and centres auditing their results

■ 325

Acute Visual Loss and Asteroid Hyalosis Associated With Posterior Vitreous Detachment

BARRY J-S, JAZAYERI F, BOOTH A
Royal Eye Infirmary, Plymouth

Purpose Asteroid hyalosis (AH) is classically described as interfering with the examiner's fundus view but not with the patient's vision. This report demonstrates that acute visual loss can apparently be caused by AH in the presence of a posterior vitreous detachment (PVD), but an alternative cause should be sought.

Methods An 85 year old lady had documented vision of 6/9 and AH with a normal fundus appearance. She presented 2 years later with acute visual loss over a period of 5 days, initially with symptoms consistent with a PVD. The visual acuity (Va) on presentation was hand movements (HM). The AH appeared so dense it prevented any view of the posterior pole, there was no associated vitreous haemorrhage. The red reflex was significantly reduced. There was no relative afferent pupil defect (RAPD).

Results B-scan ultrasonography demonstrated AH in the presence of a PVD with apparent post-vitreous haemorrhage. 3 weeks later the Va remained HM with no RAPD, the red reflex was slightly brighter. The patient was keen to be considered for vitrectomy, however her Va improved 6 weeks later to 6/12. A limited view of the posterior pole was regained; no focal pathology or haemorrhage in the post-vitreous space was seen.

Conclusion This case initially appeared to demonstrate acute visual loss with AH following a PVD. The likelihood of coexistent occult post-vitreous haemorrhage (most likely from a haemorrhagic PVD though with an extensive differential diagnosis) which did not enter the vitreous cavity and was concealed by the AH is discussed. Images are presented and the literature on AH and visual effects is reviewed. The case dramatically reinforces that asteroid hyalosis does not cause acute visual loss alone and can conceal other pathologies.

■ 326

Postsurgical cystoid macular oedema in patients in treatment with prostaglandins. Our experience

CASTEJON MA (1), BOLIVAR G (1), TEUS MA (1, 2, 3)
(1) Hospital Universitario Príncipe de Asturias, Alcalá de Henares
(2) Universidad de Alcalá, Alcalá de Henares
(3) Instituto Oftalmológico Internacional VISSUM, Madrid

Purpose To review the incidence of clinically symptomatic postsurgical cystoid macular edema (CME) in patients with glaucoma or ocular hypertension in chronic treatment with prostaglandins (PG) who underwent cataract surgery or combined cataract and glaucoma surgery.

Methods This is a prospective study of one hundred eyes that underwent cataract surgery or combined glaucoma and cataract surgery and who were under chronic hypotensive therapy with prostaglandin analogues before surgery. We evaluated the incidence of clinically symptomatic cystoid macular edema in the early postoperative period.

Results CME was present in two patients of the one hundred eyes studied. One of the patients presented the CME in both eyes. Both patients had an eventful surgery.

Conclusion The incidence of postsurgical CME in patients who undergo cataract or combined surgery previously treated with prostaglandins is low.

■ 327

Management of rhegmatogenous retinal detachment with 25-g vitrectomy

RUIZ MORENO JM (1, 2), MONTERO J (2), DE LA VEGA C (2)
(1) Department of Ophthalmology, Miguel Hernández University School of Medicine, Alicante
(2) Instituto Oftalmológico de Alicante, Vitreo-Retinal Unit, Alicante

Purpose To evaluate the efficacy and safety of 25-gauge transconjunctival vitrectomy to treat primary rhegmatogenous retinal detachment (RRD).

Methods 26 consecutive eyes from 26 patients with RRD (PVR milder than grade A) were treated by 25-gauge transconjunctival vitrectomy. Initial Best-corrected visual acuity (BCVA) and at month-6, spherical equivalent change, the number of re-detachment and complications were analyzed.

Results Anatomic re-attachment at month-6 was achieved in 88.5% after 1 procedure. In 3 cases the retina re-detached at 15 days, 1 and 2 months due to undetected break, new break or by PVR development respectively. Mean preoperative BCVA was 0.49 ± 0.39 (range, 0.01 to 1) compared to 0.61 ± 0.34 (range, 0.05 to 1) postoperative 6 months mean BCVA ($p=0.03$; Student t test paired data). No significant change in spherical equivalent was observed (from -4.0 ± 6.8 to -4.5 ± 6.9).

Conclusion Transconjunctival 25-gauge vitrectomy is effective in the management of primary rhegmatogenous retinal detachment without PVR or PVR grade A. Financial disclosure: None

■ 328

Massive suprachoroidal haemorrhage

VIDE ESCADA EICKMANN A (1), NASCIMENTO J (1), HENRIQUES J (1), QUINTAO T (1), CASTANHEIRA-DINIS A (2)
(1) Retina Department - Instituto de Oftalmologia Dr. Gama Pinto, Lisbon
(2) Instituto de Oftalmologia Dr. Gama Pinto, Lisbon

Purpose To describe the surgical management of massive expulsive suprachoroidal haemorrhage (SCH) with associated poor prognostic signs. A review of the current literature is undertaken, focusing on the available therapeutic options.

Methods Three female patients, average age 82.3 years, presented with SCH during surgeries of phacoemulsification (F), trabeculectomy (T) and extracapsular lens extraction (E). The risk and prognostic factors were evaluated and the surgical design was made, based on this data as well as on clinical examination and ultrasound findings. The average follow-up was 1.7 years.

Results A good final outcome was achieved. The second patient developed an inferior retinal detachment that was successfully treated with surgery. Visual acuities were 20/40 (secondary lens implantation is programmed), light perception (due to advanced glaucoma) and 20/100 (-2).

Conclusion In SCH each patient should be evaluated as individual case and the treatment should be personalized. Surgical decisions should be made in cases of poor prognosis. Furthermore ultrasound is an essential tool in the pre and post surgical evaluation.

■ 329

An audit of clinical indications for day-case binocular indirect laser Panretinal Photocoagulation for Diabetic eye disease

SOMANATHAN S, MUSHTAQ B, TSALOUIMAS MD
Birmingham and Midland Eye Centre, Birmingham

Purpose Laser photocoagulation can be delivered at the slit-lamp or by using the binocular indirect delivery system. An increase in the number of diabetic patients having indirect laser treatment for proliferative disease prompted us to conduct an audit to study the common clinical indications for the procedure and the efficacy of this treatment modality, in a medical retina firm at a tertiary referral centre.

Methods All patients who received indirect laser photocoagulation at the Birmingham and Midland Eye Centre between September 2004 and February 2005 were included in the study. Data was collected retrospectively from the patient records. Indications for the procedure were looked into and the main endpoints studied were change in visual acuity at the second outpatient follow-up and stabilisation of retinopathy.

Results There were 41 sessions of indirect laser treatment during the 6 month period. Thirty four eyes of 24 patients had the laser treatment of which 15 eyes had received no previous laser. An average of 1500 burns was delivered per session. The main indications for indirect laser were severe retinopathy(37%) and unsuitability for treatment at the slit-lamp (33%) due to factors such as intolerance to slit-lamp laser, patient obesity and cervical arthritis. Eighty percent of eyes had increased or stable vision after laser treatment at the second follow-up. Only 8 eyes needed further laser treatment at the slit-lamp at the end of the follow-up period.

Conclusion Indirect panretinal photocoagulation should be considered in all patients who are unsuitable for slit-lamp mode or when a large amount of laser needs to be delivered quickly to stabilise severe disease.

■ 330 / 2136

Expression of CTLA4-Ig and vIL-10 by gene-engineered dendritic cells leads to prolongation of corneal allograft survival

SCHLICKEISER S (1, 2), RITTER T (3), LI XQ (2, 4), APPELT C (1), VOLK HD (1), PLEYER U (2), SAWITZKIB (1)

(1) Institute of Medical Immunology, Charité Universitätsmedizin Berlin, Berlin

(2) Dept. of Ophthalmology, Charité Universitätsmedizin Berlin, Berlin

(3) Regenerative Medicine Institute, NUI Galway, Galway

(4) Dept. of Ophthalmology, Huazhong University of Science and Technology, Wuhan

Purpose Systemic adenoviral CTLA4-Ig or vIL-10 gene therapy has been previously shown to be successful in prevention of allograft rejection in experimental keratoplasty. To achieve long-term survival without systemic administration of adenoviral particles this study focuses on the application of ex vivo gene-modified dendritic cells (DCs).

Methods DCs were generated from murine bone marrow. Cells were transduced with an adenovirus encoding vIL-10, CTLA4-Ig or EGFP as a control. Allo-stimulatory capacity of DCs was determined by analyzing the proliferation of co-cultured allogeneic T cells. 7 days prior to experimental keratoplasty 2x10⁶ transduced BALB/c donor DCs were injected i.v. into C57BL/6 mice receiving corneal allografts. A rejection score was daily graded by cornea clarity and oedema. Intra-graft cytokine mRNA expression and presence of Foxp3+ CD25+ CD4+ regulatory T cells in lymphoid organs were analyzed by RT-PCR and flow cytometry.

Results Transduction of DCs with AdCTLA4-Ig prior to LPS stimulation dramatically diminished their allo-stimulatory capacity. Compared to controls, AdvIL-10 transduced DCs showed a significant decrease in T cell activation. Adoptive transfer of CTLA4-Ig transduced DCs resulted in a significant prolongation of graft survival, vIL-10 expressing DCs strongly reduced the rejection rate. This could be confirmed by higher levels of Foxp3+ CD25+ CD4+ regulatory T cells in lymph nodes and spleen of animals receiving CTLA4-Ig or vIL-10 transduced DCs.

Conclusion Adoptive transfer of gene-engineered tolerogenic dendritic cells is a promising approach for the prevention of corneal allograft rejection. (Supported in part by DFG PI 150/14-1/2)

■ 331 / 2137

Prolongation of corneal allograft survival by topical application of Everolimus

PLEYER U (1), BUCH G (2), OTASEVIC L (1), SCHLICKEISER S (3),
BERTELMANN E (1), LI XQ (4)

(1) Department of Ophthalmology, Charité - Universitätsmedizin Berlin, Berlin

(2) Institute of Pharmacy, Charité – Universitätsmedizin Berlin, Berlin

(3) Institute of Medical Immunology, Charité – Universitätsmedizin Berlin, Berlin

(4) Huazhong University of Science and Technology, Wuhan

Purpose Everolimus is a novel proliferation signal inhibitor with potent immunosuppressant effects that holds promise to prevent acute allograft rejection. Commonly, topical application of immune modulatory agents is preferred to its systemic use to reduce side effects of the drug. Here we evaluated the efficacy of topical administration of Everolimus on experimental corneal grafts.

Methods Female Lewis rats received 3.5 mm grafts of DA MHC I/II incompatible donors and were randomly assigned to receive either, 0.05% Everolimus in a microemulsion formulation (1), 0.025% Everolimus in a microemulsion formulation (2) or the drug vehicle (3) as control. All treatments were started at the day of surgery and applied five times daily for 35 days. Grafts were graded every day and a rejection score was generated based on cornea clarity and oedema.

Results Local administration of 0.05% or 0.025% Everolimus was effective in prolonging the mean survival time of corneal grafts (MST 21.4 ± 7.0 days, 16.4 ± 2.3 days) as compared to the vehicle control group (13.3 ± 1.7 days; p=0.006 and p=0.015).

Conclusion These data indicate that topically applied Everolimus significantly prolonged the corneal allograft survival in an experimental keratoplasty model. (Supported in part by DFG PI 150/14-2)

■ 332 / 2138

Comparison of the Visual Acuity by Time Index with conventional Survival Curves for the evaluation of osteo-odontokeratoprostheses functional results

MICHAEL R (1), TEMPRAÑO J (2), CHAROENROOK V (2), DE LA PAZ M (2), HITZL W (3), GRABNER G (3), BARRAQUER RI (2, 1)

(1) Institut Universitari Barraquer, Barcelona

(2) Centro de Oftalmología Barraquer, Barcelona

(3) Universitätsklinik für Augenheilkunde und Optometrie, Paracelsus Medizinische Privatuniversität, Salzburg

Purpose To analyse the functional results of osteo-odontokeratoprostheses comparing conventional survival analysis with the Visual Acuity by Time Index.

Methods We reviewed 180 charts of patients that underwent osteo-odontokeratoprostheses (OOKP) at the Centro de Oftalmología Barraquer. Mean follow-up time was 8.2 years (range 1 months to 30 years). Kaplan-Meier survival curves with 95% confidence interval (CI) were calculated for functional success defined as BCVA >= 0.05. Visual Acuity by Time (VAT) - Index also with 95% CI was calculated based on the Monte Carlo method. Only one operated eye per patient was included in the analysis.

Results Based on Kaplan-Meier, 5 year functional survival was 53% (CI 45 - 62%). Mean survival time was 10.5 years (CI 8.4 - 12.7 years) and median survival time (loss of 50% of cases) was 5.4 years (CI 3.6 - 7.2 years). According to the VAT-Index calculations, maximum VA can be expected at 1.2 years after surgery with a mean VA of 0.35 (CI 0.29 - 0.41). At 5 years after OOKP, mean VA of all cases was found to be 0.26 (CI 0.20 - 0.34).

Conclusion Survival analysis estimates the time point when VA drops below a predefined value (terminal event) without analysing the time course of VA. The VAT-Index can estimate the VA at a certain time points as well as average VA within arbitrary time intervals after surgery.

■ 333 / 2139

Swelling and deswelling of human corneas during eye banking: present and future

THURET G, ZHAO M, GAIN P

Ophthalmology department, EA 3063 'Cell survival and adhesion in cancers and grafts',
Faculty of medicine, Saint-Etienne

Purpose Stromal swelling is a mandatory side effect of human corneal organ culture before graft. After few days of storage, it leads to the doubling of corneal thickness, stromal clouding and dramatic Descemet folding. Causes for this stromal hyperhydration are multiple, mainly the partial loss of functional epithelial and endothelial barrier. The consequences of such a swelling, at a cellular level are not yet fully understood but the triggering of endothelial apoptosis, for instance is very likely, be it direct or indirect by the disturbance of cell adhesion to the Descemet in the folds. To reverse the swelling, corneas have to be immersed in a storage medium supplemented with a high molecular weight molecule. During this step called 'deswelling' the osmotic properties of the macromolecule contribute to extract the water excess accumulated within the stroma. To date, the only macromolecule validated for a routine clinical use is the T500 Dextran, a glucose biopolymer. This corneal deswelling immediately before graft facilitates the suture, dramatically increases the transparency and accelerates the post operative visual recovery. Nevertheless, during this step a very important endothelial cell loss is observed, varying between 7 to 15 % depending on the deswelling duration. Efforts to replace the T-Dextran have been made during the past few years, using either hydroxyethyl starch (polysaccharide) or poloxamer family (non-ionic branch copolymers of poly(ethylene oxide)/propylene oxide/ethylene oxide) with the hope to reduce the endothelial cell toxicity during the final deswelling step. Poloxamer 188 is currently evaluated in a randomized clinical trial. Another strategy consisting in using hydroxyethyl starch all along the organ culture to avoid stromal swelling from the beginning is also under evaluation.

■ 334 / 315a

Correlation between impression cytology and Labial salivary gland biopsy in Sjögren's syndrome

LANGMAN ME (1), GICQUEL JJ (2), ROBLOT P (3), GOMBERT JM (4),

DIGHIERO PL (2)

(1) St Louis Hospital Ophthalmology Department, Saint Jean d'Angély

(2) Jean Bernard University Hospital Ophthalmology Department, Poitiers

(3) Infectious Diseases / Jean Bernard University Hospital, Poitiers

(4) Immunology / Jean Bernard University Hospital, Poitiers

Purpose To evaluate a possible correlation between impression cytology (IC) and histopathological findings obtained from salivary gland biopsy in patients with Sjögren syndrome.

Methods Thirty five consecutive patients who were referred on the same day of the salivary gland biopsy, to our department for severe dry eye were enrolled and had complete ophthalmological examination including refraction, tear function testing (break up time, jones test), slit lamp photographs. Two IC specimens per eye were collected for histocytological analysis (Nelson classification) and a study in flow cytometry of the expression of HLA-DR.

Results Thirty patients out of thirty five were diagnosed with Sjögren syndrome. There is a significant correlation between the severity of the histopathological findings from salivary gland biopsies, functional study of the tear film, results obtained from IC specimens with Nelson classification and the overexpression of HLA-DR measured in flow cytometry.

Conclusion A complete ophthalmological examination associated with the study of the conjunctiva with IC may render obsolete the use of gland biopsy in Sjögren syndrome.

■ 335 / 3155

Improvement of dry eye symptoms with polyunsaturated fatty acids

GARCHER CREUZOT C (1), PASSEMARD M (1), LAFONTAINE PO (1), VIALS S (2), JOFFRE C (2), ELENA PP (3), POUILQUIEN P (4), BAUDOUIN C (5), BRON AM (1)

(1) Department of Ophthalmology, Dijon

(2) Eye and Nutrition Unit - INRA, Dijon

(3) Cliropta, Nice

(4) Thea Laboratories, Clermont-Ferrand

(5) Department of Ophthalmology - CHNO XV-XX, Paris

Purpose Polyunsaturated fatty acids (PUFA) are involved in inflammatory pathways via prostaglandins. Conjunctival inflammation is a hallmark of all dry eye syndromes. We investigated the role of dietary n-6 and n-3 fatty acids in patients suffering from ocular dryness with peculiar attention to inflammatory markers.

Methods 71 patients presenting with mild to moderate dry eye syndromes were randomly assigned to Nutrilarm® or placebo pills, twice a day for 6 months. Schirmer test, BUT, fluorescein and lissamin green stainings were performed at inclusion and after 1, 3 and 6 months. Impression cytology to evaluate MHCII antigens was harvested at D0, Month 3 and 6. Tear PGE1 levels were measured at D0, Month 1, 3 and 6. Furthermore, a questionnaire related to the dry eye symptoms and global discomfort was provided at every visit.

Results Schirmer test, BUT, fluorescein and lissamin green stainings were improved with treatment when compared to placebo but the difference remains non statistically significant. We found the same trend with MHCII and prostaglandin tear secretion as well as discomfort feeling ($p=0.091$). Efficacy evaluated by the patients and the investigator was nearly significant ($p=0.052$ and $p=0.054$ respectively). For some signs, such as reflex tearing and conjunctival hyperaemia, the improvement was nearly significant ($p=0.047$ and 0.045 , respectively). The same results were found with skin quality and emotional condition which were improved (61% with treatment vs 36% with placebo).

Conclusion This double-masked, pilot study shows that PUFA seem an interesting tool to alleviate the symptoms related to dry eye syndrome. These results should be confirmed using a larger study population.

■ 336 / 3156

Ultrastructural Conjunctival Surface Changes in Patients with Sjögren's Syndrome: Long term Results

KOLIFAKIS DI (1), KARABATSAS CH (2), SAKKAS LI (3), ALVANOU A (4), MANTHOS AK (4), CHATZOULIS DZ (2)

(1) University of Thessaly Medical School, Larissa

(2) Dept. of Ophthalmology, University Hospital of Larissa, Larissa

(3) Dept. of Rheumatology, University Hospital of Larissa, Larissa

(4) Laboratory of Histology, Embryology, Anthropology, Aristotle University of Thessaloniki, Thessaloniki

Purpose To demonstrate the ultrastructural appearance of the conjunctival surface epithelium in patients with Sjögren's syndrome (SS) compared with normal subjects.

Methods Conjunctival tissue specimens from 13 normal subjects and 32 patients with SS were obtained by bulbar conjunctival biopsy, and examined by transmission electron microscopy.

Results The average number of microvilli per 8.3 μm epithelial length was significantly lower in the SS group than that in controls (16.28 ± 5.72 vs. 28.92 ± 3.09 , $P < 0.01$). The microvillus height ($0.487 \pm 0.163 \mu\text{m}$) and height/width ratio (1.587 ± 0.49) in the conjunctival epithelium in the SS group were significantly lower than those (height: $0.939 \pm 0.093 \mu\text{m}$, $P < 0.01$; and height/width ratio: 3.740 ± 0.511 , $P < 0.01$) in normal individuals. The microvilli in the SS group were wider than those in the control group ($P = 0.046$). Furthermore, the average number of secretory vesicles (per 8.3 μm epithelial length) in the apical conjunctival epithelial cell was significantly reduced in the SS group (15.77 vesicles ± 5.77), compared to controls (33.5 vesicles ± 2.07 , $P = 0.01$). In addition, while the OSG was always present in controls, this was not detectable in all but one SS conjunctival specimens.

Conclusion The ultrastructural morphology of the apical conjunctival epithelium is altered in patients with SS. Our findings suggest that an intact OSG may play a key role in the maintenance of a healthy ocular surface possibly by preventing abrasive influences on the apical epithelial cells.

■ 337 / 3157

Addition of ganciclovir to the cornea preservation medium : kinetics and toxicity

ROBERT PY (1), SAUVAGE FL (2), THURET G (3), RANGER-ROGEZ S (4), ADENIS JP (1), ROUSSANE MC (5), GAIN P (3), MARQUET P (2)

(1) Ophthalmology, Limoges

(2) Pharmacology, Limoges

(3) Ophthalmology, Saint-Etienne

(4) Virology, Limoges

(5) Tissue Bank, Limoges

Purpose HSV-related primary endothelial necrosis has highlighted the possible transmission of HSV from donor to recipient in human keratoplasty. We investigate the pretreatment of donor cornea during preservation by addition of ganciclovir in the preservation medium.

Methods 2mg/L, 10 mg/L or 20mg/L ganciclovir is added in the preservation medium of human corneas excluded from the graft process for serological reasons. Ganciclovir concentration is tested in preservation medium and in corneal tissue every 8 days for 3 weeks. Corneal toxicity is assessed by trypan blue staining.

Results Ganciclovir concentration undergo linear decrease during preservation, but remains above CI 50 fo HSV after 3 weeks in all cases. Corneal transparency remains normal during the whole process. Endothelial toxicity is discussed.

Conclusion Ganciclovir supplementation of preservation medium will increase viral security of corneal grafts. This is the first step in the way to certification and in vivo experiments.

■ 338 / 3158

Bevacizumab (Avastin) inhibits inflammatory hem- and lymphangiogenesis in the cornea

BOCK FB, ONDERKA J, DIETRICH T, BACHMANN B, KRUSE FE, CURSIEFEN C
Department of Ophthalmology, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen

Purpose Intention of this work was to analyze whether Bevacizumab can inhibit inflammatory angiogenesis and in addition also lymphangiogenesis in the cornea. Bevacizumab (Avastin^{*}) is a recombinant, humanized, monoclonal antibody against VEGF-A, FDA-approved for the treatment of colon carcinomas in 2004.

Methods The mouse model of suture induced corneal neovascularization was used to assess the antihem- and antilymphangiogenic effect of Bevacizumab by systemic and topical application. Corneal flat mounts were stained with LYVE-1 as a specific lymphatic vascular endothelial marker and CD31 as panendothelial marker and blood- and lymph-vascularised areas were analyzed morphometrically. The inhibitory effect of Bevacizumab on lymphatic endothelial cells (LEC) was analyzed with a colorimetric (BrdU) proliferation ELISA. The binding capacity of Bevacizumab to murine VEGF-A was analyzed using Western Blot.

Results The systemic/topical application of Bevacizumab significantly inhibited the outgrowth of blood ($p<0.006/p<0.001$) and lymphatic ($p<0.002/p<0.0001$) vessels. Inhibition of the proliferation of LECs was also significant ($p<0.0001$). Western Blot analysis showed, that Bevacizumab binds to both human as well as murine VEGF-A.

Conclusion Bevacizumab inhibits both inflammation-induced angiogenesis as well as lymphangiogenesis in the cornea by topical as well as systemic application. This suggests an important role of VEGF-A in corneal lymphangiogenesis. Bevacizumab may be useful in preventing immune rejections after penetrating keratoplasty or tumor metastasis via lymphatic vessels.

■ 339 / 3159

Use of Platelet-Rich Plasma In The Treatment of Ocular Surface Syndrome Following Laser In Situ Keratomileusis (LASIK)

PASTOR S (1), RUIZ-COLECHA J (2), RODRIGUEZ A (1), ARTOLA A (2), ALIO JL (2)

(1) Research, Development and Innovation. Instituto Oftalmológico, Alicante

(2) Ophthalmology. Instituto Oftalmológico, Alicante

Purpose To treat patients suffering from ocular surface syndrome following Laser in situ Keratomileusis (LASIK) with Platelet-Rich Plasma.

Methods Autologous PRP was used in a consecutive series of 20 cases with significant dry eye symptoms following corneal refractive surgery with punctate keratitis at the slit lamp examination. Selected cases were followed by impression cytology.

Results Autologous PRP was useful and effective in decreasing subjective symptoms and improving visual performance in 50% of the cases, 25% showed moderate improvement, improving also the slit lamp appearance, and in 20% of the cases the treatment was ineffective. The study of selected cases by impression cytology showed that significant changes appeared in the cytology pattern following 1 month, and especially 2 months of treatment.

Conclusion Autologous PRP might be a useful tool in the management of cases of ocular surface syndrome following LASIK.

■ 340 / 3355

NGF promotes the healing of bilateral recurrent corneal erosion in dogs

DODI PL (1), COSTA N (2), ALOE L (3)

(1) Dept. of Animal Health - University of Parma, Parma

(2) Dept. of Pharmacy Sciences - University of "Magna Grecia", Catanzaro

(3) Inst. of Neurobiology and Molecular Medicine - CNR EBRI, Rome

Purpose To report a case of bilateral recurrent corneal erosion treated with Nerve Growth Factor.

Methods A 6-year-old female French Bulldog with a bilateral recurrent ulcer covering over 80% of the right eye surface and 40% of the left one. Several treatments were tried over a 5 weeks period without any improvement. Before NGF treatment cell debris were removed using a dry cotton swab to encourage epithelial attachment; the corneal erosion was topically treated with purified murine NGF diluted in paraffin oil (value: 50 microgram/ml) for 4 weeks (2 drops every 12 hours).

Results Four days after NGF treatment the ulcers showed neurovascolorization with size reduction. After 2 weeks the cornea was negative to fluorescein, the central leucoma was vascularized. A further 3 weeks review showed the leucoma was slightly evident; at the end of the therapy the cornea showed a significant improvement of transparency.

Conclusion This report showed that bilateral recurrent corneal erosion in a French Bulldog was successfully healed with topical application of NGF. This result confirmed and extended previous evidences on human cornea's ulcers. NGF caused also a marked anti-inflammatory action of the dog's cornea and a "remodelling" action of the corneal epithelium. The present findings suggest a therapeutic potentiality of NGF, particularly when other available therapies fail to promote healing.

■ 341 / 3356

Towards the Development of Purpose-designed Ocular Bandage Lenses

BATEMAN S, MANN A, TIGHE BJ

Biomaterials Research Unit, Aston University, Birmingham

Purpose This poster is concerned with recognized analogies between the cornea & chronic wound sites as the basis for rational materials design of bandage lenses for ocular wounds.

Methods Macromer technology has enabled a common synthetic base to be used for fabrication of hydrogel-based contact lens materials & wound dressings. Nelfilcon functionalised PVA (Ciba Vision) used in conjunction with purpose-designed macromers enables the fixed charge density & functional group balance to be approximately equated to wound tissue. The materials are applied to the bed of chronic slow/non-healing ulcerated wounds, wound fluid is sampled for analysis & the state of healing of the wound noted. Removed dressings are retained for analysis. A similar nelfilcon macromer base is used as the basis for fabrication of ocular bandage lenses.

Results Initial clinical results show enhanced healing when there is ionic similarity of hydrogel surface & wound tissue. Microlitre-scale assays for a series of biochemical markers have been established and are used to monitor progress of wounds. Similar markers are found in the contact lens-wearing eye and, importantly, some (eg vitronectin) are predominantly associated with the posterior surface, and others (eg kininogen) with the anterior surface. The elimination or down-regulation of certain biochemical moieties (eg bradykinin), coupled with the normal accumulation of other factors (eg vitronectin) are clearly important to enhance ocular healing.

Conclusion Parallel studies on wound dressing & contact lens materials & the biochemistry of these two body sites are providing useful insight into the design of materials for ocular bandage lenses which provide a more effective healing environment than current cosmetic contact lenses.

■ 342 / 3357

Early limbal stem cell deficiency in KID Syndrome

LANGMAN ME (1, 2), GICQUEL JJ (3), MILIN S (4), LEVILLAIN PM (4), MESNIL M (5), DIGHIERO PL (3)

(1) St Louis Hospital / Ophthalmology Department, St Jean d'Angély

(2) Jean Bernard University Hospital / Ophthalmology Department, Poitiers

(3) Ophthalmology / Jean Bernard University Hospital, Poitiers

(4) Pathology / Jean Bernard University Hospital, Poitiers

(5) UFR Sciences Fondamentales et Appliquées / Physiology, Poitiers

Purpose To present one case of early limbal stem cell deficiency in a seven year-old boy with authentified KID syndrome (isolated mutation D50N of the GJB2 gene).

Methods The boy was referred to our department by his dermatologist for systematic ophthalmological examination. Refraction, slit lamp examination, tear film study with fluorescein, maebomian secretions examination and impression cytology (IC) were performed. Two IC specimens were collected for each eye; one for Nelson classification, the other for the study of the expression of HLA-DR in flow cytometry.

Results Slit lamp examination revealed a calm white eye. The cornea limited peripheral stromal invagination on 360° in both eyes with no preponderance for contact areas with the eyelids. Break up time was normal OS. No maebomian dysfunction was found. Nelson score confirmed limbal stem cell deficiency while flow cytometry showed no overexpression of HLA-DR compared with data obtained from normal subjects.

Conclusion Limbal stem cell deficiency can be found in young patients with KID syndrome. It seems not due to chronic ocular surface inflammation nor maebomian dysfunction but to primary limbal stem cell dysfunction caused by the mutation of the connexin 26 gene.

■ 343 / 3454

In vivo interferometric measurements of the tear film stability on soft contact lenses

SZCZESNA DH (1), STENEVI U (2), KASPRZAK H (1)

(1) Institute of Physics, Wroclaw University of Technology, Wroclaw

(2) Department of Ophthalmology, Sahlgren University Hospital, Mölndal

Purpose The purpose of this study was to measure the stability of the precontact lens tear film on different types of soft contact lenses.

Methods We applied a non-invasive, interferometric method – Lateral Shearing Technique for *in vivo* investigating of the smoothness of external layer of the tear film and its stability (TFS) in time. Interferometry allows dynamic measurements of the TFS in real time by observation of the regularity of interference fringe pattern. Evaporation of tears, their instability and appearance of the breakups cause changes in fringe regularity. Fast Fourier Transform has been used for quantitative assessment of the fringe smoothness and the parameter M2 was introduced for quantitative description of the smoothness of the tear film. This parameter is lower for a smooth and higher for irregular surface of the tear film.

Results We examined three different groups of contact lenses: daily, two-weekly and monthly. All examined twenty subjects had no problems with the tear film. The stability of the tear film was examined before the contact lens wear, 5 minutes after contact lens morning application, the same day in the afternoon and two weeks later. In majority cases (69%) the tear film smoothness on the contact lens was directly correlated with the tear film quality on the cornea. The tear film is more stable between blinks on daily than on monthly contact lenses. The tear film is less stable at the beginning of the contact lenses wear and more stable in afternoon or after two weeks.

Conclusion The method is characterised by a high accuracy and sensitivity. Evident differences can be demonstrated in tear film quality on soft contact lenses.

■ 344 / 3455

Oxygen Transmission Characteristics of a Silicone Hydrogel Lens in Custom Parameters

GILES TG

CIBA Vision, Duluth

Purpose The introduction of silicone hydrogel lenses using high volume manufacturing has made this technology accessible to the majority of more than 89 million estimated contact lens wearers worldwide. There remains however an important sub-segment of the population for whom silicone hydrogel lenses are not available. This group consists of several million individuals who require extreme parameters, ranging from high plus and high minus powers to high cylinder powers and to designs which are flatter or steeper as well as larger or smaller than typical cast molded lens parameters.

Methods Silicone hydrogel prototype lenses have been produced in an 8.4 mm base curve and 14.0 mm diameter in powers of +16.00 DS, -16.00 DS, -3.00 DS and -3.00/-2.25 @ 090. Using a Lens Thickness Profiler, lens thickness across the entire surface of each lens was measured and the Dk/t calculated and plotted. This procedure was repeated with traditional hydrogel materials (phemifilcon A 38% water and methafilcon B 55% water) in the same power with similar parameters.

Results The Dk/t maps demonstrate the enhanced oxygen transmission profile for the silicone hydrogel material compared with traditional hydrogel materials. This is especially evident in the high plus and high minus designs, where lens thickness increases considerably.

Conclusion Modern silicone hydrogels have dramatically transformed the landscape of contact lens practice in the 21st century and clinical studies have shown fewer hypoxic signs among wearers of silicone hydrogel wearers. The availability of these materials in expanded, made-to-order parameters will allow practitioners to satisfy a previously unmet need among the patient population which stands to benefit the most from an enhanced oxygen profile.

■ 345 / 3456

Solutions, Contact Lenses & Wear: Then & Now

FRANKLIN VJ, TIGHE BJ

Biomaterials Research Unit, Aston University, Birmingham

Purpose Since the introduction of corneal contact lenses over fifty years ago, clinicians have sought & developed solutions, which promote or enhance lens comfort upon insertion & during wear. These have evolved from the relatively simple wetting solutions used with rigid polymethyl methacrylate to the highly sophisticated multi-purpose solutions used the current generation of contact lens materials. The underlying problem in the design of ophthalmic biomaterials continues to be that of ocular compatibility, although our understanding of the underlying biological issues involved has become progressively more sophisticated. Legislation, new cleaning regimes, contact lens materials & wear modalities all have had an impact on the perceived clinical performance & acceptability.

Methods This poster summarises some of the developmental issues & properties of the components of contact lens solutions & the impact of different wear modalities & contact lens materials on the use of such materials in the ocular environment, paying particular attention to the changes that have been seen in the last decade.

Results Taken together the properties of the solutions described provide a basis for understanding & interpreting the clinical behaviour of ophthalmic biomaterials used for contact lens applications, especially the solutions sensitivity observed with the new generation of silicone hydrogel lenses.

Conclusion Reintroduction of silicone hydrogels for contact lens applications & recent issues relating to compatibility with contact lens solutions has highlighted the complexity of ophthalmic biomaterials & the need to develop specific material & solution combinations for optimal ophthalmic performance.

■ 346 / 3457

Microfluidic Technology for Routine Tear Analysis

MANN A, TIGHE BJ

Biomaterials Research Unit, Birmingham

Purpose To study and obtain individual tear protein profiles by monitoring the effects of contact lens wear and factors affecting the normal ocular response and to assess the use of the contact lenses as a probe and means of sampling the tear film. The use of tears as a non-invasive diagnostic medium has great clinical potential.

Methods This work employed a fully automated chip based protein separation assay that is vastly superior and more convenient than comparable conventional gel electrophoresis methods. 4ml of tears were collected from a variety of patients in a series of self-controlled, clinically managed, studies. The tear envelope that is attached by surface forces to the contact lenses at the time of removal was also evaluated. All samples were assayed on a 2100 Agilent Bioanalyzer with Protein Lab Chip' kits.

Results These studies investigated tear proteins in the range of 14-200kDa paying particular attention to the relative concentrations of lysozyme, lipocalin, sIgA, IgG and lactoferrin, in conjunction with overall tear electropherogram 'fingerprint'. Distinct protein compositional changes were observed between the lens and non-lens wearing eye, and the normal and compromised eye. In all 15 distinct peaks were detected, some of the peaks were found in only a small number of subjects or on a single occasion over the sampling period. Interestingly a peak at 16kDa which was not typically observed in normal basal tears, was common to the lens wearing tear and tear envelope.

Conclusion Laboratory technology is progressing towards miniaturisation and greater automation through the development of lab-on-a-chip platforms. These may in the future become part of routine clinical practice, thus incorporating diagnostic procedures into normal patient care.

■ 347 / 3458

Measurement of Frictional Characteristics of Contact Lenses

ROSS G, TIGHE BJ

Biomaterials Research Unit, Aston University, Birmingham

Purpose Biotribology is the study of the lubrication, friction & wear at biological interfaces. When a contact lens is placed on the eye, the surface of the contact lens will influence the motion between the eyelid & the surface of the eye & this influences lens comfort. This project is driven by the need to establish in vitro techniques for measurement of this important aspect of the interaction between contact lenses and the anterior eye.

Methods A high sensitivity tribometer has been identified & adapted for the study of contact lenses. The lens is placed on a convex mould, which slides against a moving substrate (which may be varied) in the optional presence of an appropriate lubricating solution. The resistance to motion is expressed in terms of the coefficient of friction. The whole apparatus sits on an air table to isolate it from vibration. Using this instrument, coefficients of friction down to 0.005 can be measured reproducibly.

Results Three distinct factors can be discerned that affect measurements made in vitro using this technique: bulk mechanical properties & the surface chemistry of the lens; nature of the lubricating layer; the hydration of the lens, & the deposition & subsequent degradation of tear components. Current silicone hydrogels show interesting individual 'friction fingerprints'.

Conclusion The technique shows clear material-dependant differences in the values of friction coefficient of different lens materials of unworn lenses & also changes in the frictional behaviour of lenses as a consequence of wear. It provides an additional tool in the study of the complex interactions that affect patient-to-patient & diurnal variations in comfort.

■ 348

Corneal morphology, topography and sensitivity in a family with inherited recurrent corneal erosions – Dystrophia Helsingiensis

NEIRA W (1), LINDBOHM N (1), TUISKU I (1), HAMMAR B (2), DELLBY A (2), KONTTINEN L (2), TERVO T (1), FAGERHOLM P (2)

(1) Department of Ophthalmology, University of Helsinki, Helsinki

(2) Department of Ophthalmology, Linköping Universitetshospital, Linköping

Purpose To describe morphological parameters in healthy and in affected family members - with inherited recurrent corneal erosions - by examining the cornea with corneal confocal microscopy (CCM), corneal computarized topography (CCT) and Belmonte noncontact gas esthesiometer (NE).

Methods Seven family members, 5 affected and 2 unaffected were examined. The affected members represented the different stages of corneal changes from a normal cornea to fibrosis of the central cornea and discrete bumps. To evaluate the corneal morphology and nerve structure, the patients were examined under a CCM (ConfoScan 3. Corneal confocal Microscope Nidek Technologies. Software Version 3.4). CCT data were collected using a Tomey, TMS – 2N Topographic Modelling System (software: version 2.4.2)). Corneal mechanical sensitivity was measured with modified NE.

Results CCM showed changes at the epithelium, stroma and sub basal nerve plexus. Except for one eye of the affected members, all the CCT, -even controls- showed irregular astigmatism. Affected individuals had a decreased corneal sensitivity. There was no correlation between the patients' ages and corneal sensitivity.

Conclusion CCM is an effective tool to evaluate corneal morphology in this hereditary anterior corneal dystrophy. Correlation between irregular CCT and CCM findings in control patients was not found. NE revealed a possible slight reduction in corneal sensitivity in the affected members.

■ 349

Regulation of limbal stem cell growth and differentiation by factors produced by the wounded cornea

HOLAN V (1), KRULOVA M (1), TAVANDZI U (2), POKORNA K (1), ZAJICOVA A (1), FILIPEC M (2)

(1) Institute of Molecular Genetics, Prague

(2) Eye Laboratory, Charles University, Prague

Purpose Purpose. To investigate signaling molecules produced by wounded cornea which regulate the growth and differentiation of mouse limbal stem cells (LSC).

Methods Methods. Epithelial layer of the corneas was mechanically damaged and the corneas were removed at different time intervals after the injury and cultivated for 24 or 48 h. The supernatants were tested for the ability to modulate the growth and differentiation of LSC which were enriched by gradient centrifugation on Percoll or were sorted as a side population. The expression of genes for various cytokines and growth factors in wounded corneas or changes in the expression of selective limbal (ABCG2, keratin 19) or corneal (keratin 12, connexin 43) cell markers were determined by RT-PCR.

Results Results. Cells from limbuses excised 24 h after the injury of the cornea proliferated more vigorously than limbal cells from healthy eyes. However, 4 days after the injury the proliferation of limbal cells was significantly depressed in comparison with cells from healthy controls. Similar effects on limbal cell proliferation had supernatants from the cultures of damaged corneas. Expression of genes for numerous cytokines and growth factors was significantly enhanced in the corneas and limbuses shortly after injury. Cytokine-induced activation of limbal cells was associated with a loss of limbal cell markers and with appearance of markers characteristic for corneal cells.

Conclusion Conclusion. The growth and differentiation of LSC are regulated in both positive and negative manner by cytokines and growth factors produced by wounded cornea. These molecules are responsible for a rapid limbal cell activation and consequent corneal healing.

■ 351

Mitosis in the endothelium of donor corneas

SLETTEDAL JK (1), BERAKIK (2), RAMSTAD H (1), NICOLAISSEN B (1)

(1) Ophthalmology, Center for Eye Research, Ullevaal University Hospital, Oslo

(2) Pathology, Ullevaal University Hospital, Oslo

Purpose We wanted to study the endothelium in donor corneas with extended post-mortem time for reparative changes in an eye bank organ culture storage system.

Methods Methods Donor corneas with post-mortem time from 14 to 163 hours were obtained and stored in organ culture at 32°C for 3 and 6 days. Examination was performed with light microscopy, scanning and transmission electron microscopy. BrdU-staining was performed in cross sections of cornea and flat mount preparations of the endothelium.

Results Results All donor corneas acquired an endothelial covering after 3 days of organ culture. Light microscopy and scanning electron microscopy showed possible cell division of endothelial cells. BrdU staining showed positive cells to a variable degree, and in some of the specimens more than half of the endothelial cells were stained.

Conclusion Conclusion We demonstrate survival of endothelial cells and regeneration of the endothelial monolayer in donor corneas with extended post-mortem time up to 7 days. The results further suggest that repair of the endothelial layer in donor corneas also occurs by mitosis, not only by cell enlargement and sliding, in order to cover Descemet's membrane.

■ 350

Soluble Fas in tear fluid of patients with cystic fibrosis

MRLIGACZ M (1), ZELAZOWSKA-RUTKOWSKA BEATA (2),

BAKUNOWICZ-LAZARCZYKA (1), WYSOCKA J (2)

(1) Pediatric Ophthalmology Medical University, Bialystok

(2) Pediatric Laboratory Diagnostic Medical University, Bialystok

Purpose Cystic fibrosis (CF) is a genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene and defective expression of CFTR protein in epithelial cells, including the eye. The pathogenesis of ocular changes in CF is still unknown but CF belongs to the keratoconjunctivitis sicca, that develop in dry eye syndrome. The causes of dry eye are multifactorial and can be related to perturbations in the apoptotic process. In this study we examined tear fluid levels of soluble Fas (sFas) in cystic fibrosis patients.

Methods Tear samples were collected from twenty CF patients and twenty controls. Tear fluid sFas concentrations were determined by immunoenzymatic assay ELISA.

Results The mean sFas concentration was 248.63 pg/ml in CF patients and 95.69 pg/ml in control group. We found statistically increased levels of sFas in tear fluid of patients with cystic fibrosis compared with controls.

Conclusion Our findings suggest that the apoptosis appears to have a role in the pathogenesis of the ocular changes in CF patients. Tear fluid level of sFas may be a marker of the apoptotic status in these patients. The above results are important for the choice of the strategy of the treatment.

■ 352

PDT of corneal neovessels using a new hydrosoluble photosensitizer (WST11)

BOLIRGES J-L (1, 2, 3), BENEZRA D (4), BEJJANI RA (1), SAVOLDELLI M (5), JEANNY JC (1), BLANC D (6), BEHAR-COHEN F (1, 5)

(1) INSERM U598, Paris

(2) Rene Descartes University, Paris 5

(3) Ophthalmology, Hotel-Dieu Hospital, APHP, Paris

(4) Hadassah Hospital, Jerusalem

(5) Rene Descartes University, Paris

(6) Negma Lerads, Toussus le Noble

Purpose To evaluate the photodynamic treatment (PDT) potential and safety of a new hydrosoluble photosensitizer (WST11) on corneal neovascularization (NV).

Methods Methods NV was induced in rabbit corneas by lipopolysaccharide intrastromal implantation(n=6/condition). PDT successively combined 5 or 10mg/Kg of systemic WST11 (WST5/WST10), a 3.5mm, 753nm, 600mW/cm² laser beam with 100 or 150/J/cm² laser fluences (L100/L150) and a 1 or 5 min distance to light illumination (DLI1/DL15). The treated area was clinically examined. The eyes were enucleated after sacrifice at 2H, 2 and 8 days for histology analysis.

Results Results A bleaching surrounded by a ring of relative NV dilation was observed in the treated area during the PDT. Immediately after, partial NV occlusion was observed in 33% of the eyes receiving L100, WST5, DL11, only in 15% with DLI 5 and in respectively 100% and 66% of the eyes receiving L100, WST 10, DLI1 or DLI5. Total NV occlusion occurred in all eyes with L150, WST10, DL11 and in 75% of the eyes with DLI5. No corneal or ocular structure toxicity, inflammation or intraocular hemorrhages occurred after PDT.

Conclusion Conclusion 2H after PDT, histology shown vascular stasis with alterations of the NV endothelial cells without vessel wall rupture. On day 2 and 8, NV occlusion persisted in the eyes treated with the highest parameters but, on day 8, patent vessels were observed within the previously occluded NV zone. No collateral lesion of the adjacent corneal tissues was observed.PDT effect of the new photosensitizer WST11 is closely associated with the DLI probably due to its rapid elimination from the circulation. Selective PDT parameters with WST11 result in an efficient, safe and continuous occlusion of corneal NV.

■ 353

Increased expression of matrix metalloproteinases 1, 2 and 9 in human corneal grafts during corneal melting

JUKLOVA K, FILIPEC M, JIRSOVA K

Laboratory and Ocular Tissue Bank, General Teaching Hospital and Charles University Prague

Purpose To compare the presence of matrix metalloproteinases (MMPs) 1, 2 and 9 in human corneal grafts obtained during penetrating keratoplasty for corneal melting with normal human corneas.

Methods Four control corneal buttons and seven corneas obtained during penetrating keratoplasty of patients suffering from the melting of grafted corneas were used. All patients suffered from rheumatoid arthritis, four of them from Sjögren's syndrome, two from peripheral ulcerative keratitis, and one from keratoconjunctivitis sicca. Cryosections were fixed and indirect enzyme immunohistochemistry was performed using antibodies against MMP1, 2 and 9. The intensity of the signal was assessed under a light microscope (20x) using 4 grades: 0 – no staining, 1 – weak, 2 – moderate, 3 – intense staining.

Results Weak or moderate MMP2 positivity was observed in the anterior stroma and epithelium, respectively, of all control corneas. Moderate staining for MMP1 and 9 and very intensive staining for MMP2 was found in epithelial fragments of melted specimens. Moderate MMP1 positivity and intensive staining for MMP2 and 9 were observed in the anterior stroma of all melted grafts. Weak to moderate positivity for MMP1, 2, and 9 was detected in the posterior stroma of all melted grafts.

Conclusion The exact mechanism of keratolysis has not yet been clarified. The increased presence of MMP1, 2 and 9 in melted corneal grafts suggests that these enzymes may be responsible for the destruction of the extracellular matrix in grafts undergoing keratolysis.

■ 354

Refractive changes after LASIK corneal flap formation in corneas after penetrating keratoplasty

PODKOCHY A, FAGERHOLM P

Linköping University Hospital, Linköping

Purpose LASIK after penetrating keratoplasty (PKP) is not always a predictable procedure. The purpose of this study was to evaluate the refractive effect of corneal flap formation during the LASIK procedure.

Methods Fifteen eyes of 14 patients after PKP were selected for LASIK to correct myopia, hyperopia, and astigmatism in simple and combined forms. The indication for LASIK was anisometropia and contact lens intolerance. Mean preoperative myopia was -6.0 D (SD 4.75 D), mean hyperopia +3.0 D (SD 0.4 D), and mean astigmatism was -5.3 D (SD 2.25 D). LASIK was performed in two separate steps. The first step was the formation of a corneal flap alone with Hansatome microkeratome. The second step – lifting of the flap and following laser treatment with Technolas 217 z laser – was performed 1 month later. The refraction was measured before the first step and before the second step respectively. The laser treatment was performed according the refraction measurement before the second step.

Results Eight of 15 eyes (53%) showed changed refraction 1 month after the corneal flap formation. One eye developed higher myopia than before (change 3.0 D). Two eyes developed reduction of myopia (change 2.0 D in both eyes). Three eyes developed progression of astigmatism (respectively, 2, 3.5, and 6 D higher astigmatism). One eye developed reduction of astigmatism (change 2 D). In 4 eyes astigmatism changed its axis more than 20 degrees (mean change 29 degrees, SD 12 degrees).

Conclusion The formation of the corneal flap during LASIK procedure creates refractive changes in PKP corneas. LASIK performed in two separate steps may help to avoid a wrong refractive laser treatment.

■ 355

Biglycan protects keratocytes from apoptosis

KOLILIKOVSKA M, FAGERHOLM P

Linköping University Hospital, 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 involvement of biglycan in the corneal repair process. In the present study, we have investigated possible involvement of biglycan in the modulation of the survival of keratocytes.

Methods Keratocyte death was induced in cell culture by IL-1 under serum-free conditions in the presence or absence of biglycan. Histone-associated DNA fragments were assayed by using a cell death detection ELISA. For further differentiation between apoptosis and necrosis, keratocytes were stained using the annexin V-Cy3 apoptosis detection kit.

Results Quantification of histone-associated DNA fragments by the cell death detection ELISA showed that biglycan strongly protected keratocytes from dying. Apoptotic death of keratocytes dominated after the addition of IL-1. Coincubation with biglycan markedly reduced the number of apoptotic keratocytes.

Conclusion Biglycan inhibits IL-1-induced apoptosis of native keratocytes. Further studies, however, are needed to determine how biglycan might influence apoptosis.

■ 356

Inhibition of inflammatory corneal lymphangiogenesis by an anti-VEGFR3 antibody (mF4-31C1)BOCK FB, ODERKA J, DIETRICH T, BACHMANN B, KRUSE FE, CURSIEFEN C
Department of Ophthalmology, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen

Purpose Aim of this study was to analyze whether the blocking anti-VEGFR3 antibody mF4-31C1 is able to inhibit the outgrowth of pathologic new lymphatic vessels in a mouse model of suture-induced, inflammatory corneal neovascularization. In a mouse model of spontaneous, non-inflammatory corneal hem- and lymphangiogenesis the potency of this antibody has been recently demonstrated.

Methods The mouse model of suture induced corneal neovascularization was used to assess the antihem- and antilymphangiogenic effect of mF4-31C1 by systemic application. The treatment group (n=9) received mF4-31C1 intraperitoneally at day of surgery and three days later (0.5 mg/mouse). Control mice received an equal amount of control IgG solution (Jackson ImmunoResearch, Inc. CA, USA). Corneal flat mounts were stained with LYVE-1 as a specific lymphatic vascular endothelial marker and CD31 as panendothelial marker and blood- and lymph-vascularised areas were analyzed morphometrically.

Results The F4-31C1 antibody treated mice displayed nearly complete inhibition of lymphangiogenesis compared with IgG controls ($p < 0.006$). There was no significant effect on hemangiogenesis observable.

Conclusion Inflammatory corneal lymphangiogenesis seems to depend on VEGFR3-signalling. By blocking this lymphangiogenic receptor the ingrowths of lymph vessels can be inhibited almost completely. This specific anti-VEGFR3 antibody could be used to inhibit lymphangiogenesis after keratoplasty to reduce neovascularization and thereby promote graft survival.

■ 357

Clinical Practice of Consultant Ophthalmologists in Treating Herpetic Eye Disease in the United Kingdom

ZIAHOSSEINI K, MORGAN LH, IKRAM K
Stockport Eye Centre, Stockport

Purpose Herpes Simplex virus remains a leading cause of chronic ocular infection and unilateral blindness. This study evaluates the current practice of management of herpetic eye disease as there is no similar studies previously done in the U.K.

Methods A 12- question circular was posted to 903 consultant ophthalmologists. It evaluated treatment pattern of primary and recurrent epithelial and stromal keratitis and iridocyclitis.

Results Treatment strategies were more uniform than expected, agreeing with HEDS guidelines in the treatment of epithelial and stromal keratitis, but showing deviation in the use of antiviral agents for recurrent disease.

Conclusion UK clinical practice for treating herpetic eye disease is consistent but deviates from HEDS guidelines in some areas. Increased awareness of HEDS study data could address this issue

■ 358

Traumatic wound dehiscence following penetrating keratoplasty: clinical outcomes and in vivo confocal microscopy

MALANDRINI A, BALESTRAZZI A, FRUSCHELLI M, TOSI GM, CAPOROSSI A
Ophthalmology and Neurosurgery University of Siena, Siena

Purpose To evaluate clinical characteristics, management, graft survival and in vivo confocal microscopy for traumatic wound rupture after penetrating keratoplasty.

Methods From the files of the Department of Ophthalmology of the University of Siena (1998-2005), 13 patients who suffered surgical wound dehiscence after penetrating keratoplasty were included in this retrospective study. Wound rupture was managed with primary surgical closure in all patients. Visual acuity, IOP and in vivo confocal microscopy (Heidelberg Retinal Tomograph II- Rostock Cornea Module) were evaluated in the follow up.

Results Latency between corneal grafting and trauma was 8.23 years (range 4 months-38 years). Globe rupture occurred at the graft-host junction in all patients. Eight eyes (61.5%) presented a prolapsed iris. Seven patients (53.8%) presented lens or IOL expulsion. Nine patients (69.2%) presented a prolapsed vitreous. Of the ruptured eyes, 6 (46.1%) achieved a final visual acuity of at least 20/200 and only 2 (15.4%) achieved at least 20/35. Using in vivo confocal microscopy, morphological changes were detected at the graft-host junction and in the donor cornea in all cases. The morphological changes include epithelial irregularities, activated keratocytes and Langherans cells infiltration and irregular stromal fibrosis.

Conclusion Traumatic wound dehiscence is a serious and not very rare complication after penetrating keratoplasty. Prognosis and visual results are related to wound extension, posterior segment involvement and prolapse of iris, lens or vitreous.

■ 359

Novel Ocular Lubricants Based On Polymer-Phospholipid Recombinants & Hyaluronic Acid

RASUL N, TIGHE BJ
Biomaterials Research Unit, Aston University, Birmingham

Purpose The body uses hyaluronic acid (HA) and polymer-phospholipid complexes in lubrication (e.g. in articular joints) and HA-based formulations are finding increasing ocular applications. This poster examines the potential use of polymer-phospholipid recombinants combined with hyaluronic acid as ophthalmic lubricants.

Methods The rheological behaviour of the lubricant was measured on a Bohlin CV050 rheometer using a cone (10) and plate (20 mm) at 37°C. Frictional behaviour was studied on a CSM Nanoscratch tribometer using a hydrogel contact lens in conjunction with the lubricant over a sliding distance of 20mm with a smooth, moderate surface energy substrate. The surface activity of the lubricants was studied using a Langmuir trough and the static surface tension measured using a wetting balance.

Results Solutions of 0.4 % (w/v) HA mimic the reported rheological behaviour of tears. HA from the human umbilical cord showed a lower coefficient of friction (< 0.05) than conventional HA-based artificial tears when studied in combination with a hydrogel contact lens and a smooth substrate. The polymer-phospholipid complexes - based on Poly (maleic acid-alt-styrene) and dilauroylphosphatidylcholine / dipalmitoylphosphatidylcholine - greatly enhance surface activity; under repetitive expansion and compression they exhibit a dynamic surface tension similar to that of lung surfactant (< 5 mNm⁻¹).

Conclusion The combined HA and polymer-phospholipid complexes possess a balance of surface activity, rheological and biotribology properties, that are composition-dependant and show potential advantage as an artificial tear.

■ 360

Comparison between different test used to evaluate the quality and the quantity of the tear film

GONZALEZ MARTINEZ M, VIDAL LOPEZ J, SOBRADO CALVO P, JAVALOYES MORENO B
Ophthalmology, Murcia

Purpose In this study a series of tests were done to evaluate the quality and the quantity of the precorneal tear film in a group of students, and the relation between these tests was investigated.

Methods The tests were performed in 54 university students (108 eyes). To evaluate the stability of the precorneal tear film two tests were used: invasive break-up time (BUT) and noninvasive break-up time (NIBUT). The quantity of tear secretion was evaluated using the Schirmer test (ST) and the phenol red thread test (PhRT).

Results The results obtained by the cited tests were: for the BUT, the mean value was 10.95±4.50 seconds; for the NIBUT, the mean value was 12.86±7.29 seconds; for the ST, the mean value was 18.06±6.18 mm; the mean value for the PhRT test was 21.16±7.29 mm. When the BUT and NIBUT were compared, a significant correlation was obtained ($r=0.47$, $p<0.001$). When ST was compared with PhRT test, a high and significant correlation was obtained ($r=0.732$, $p<0.001$). When the ST was compared with the BUT and NIBUT tests, significant correlations were obtained (ST-BUT: $r=0.19$, $p=0.04$; ST-NIBUT: $r=0.21$, $p=0.04$), whereas with the PhRT test there was not a significant correlation (PhRT-BUT: $r=0.17$, $p=0.15$; PhRT-NIBUT: $r=0.09$, $p=0.47$).

Conclusion There is a significant correlation between the tests used to evaluate the quantity and the quality of the precorneal tear film. Moreover, the ST is statistically correlated with the tests used to evaluate the stability of the precorneal tear film, whereas the PhRT test is not correlated with them.

■ 361

**Cross – Infection and Contact Ophthalmic Devices:
Clinical Trials of a Disposable Ophthalmic Barrier System**

TIGHE BJ (1), FRANKLIN VJ (1), HENLEY CE (1), KERR J (2), GEE H (3),
WOLFFSOHN J (4), FLANAGAN J (5)

(1) Biomaterials Research Unit, Aston University, Birmingham

(2) Mayday University Hospital, Croydon

(3) First Water Ramsbury Ltd, Hilldrop Lane, Ramsbury

(4) Optometry & Vision Sciences, Aston University, Birmingham

(5) School of Optometry, University of Waterloo, Waterloo, Canada

Purpose It's 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. A Department of Health funded project has developed a four-layer barrier system consists of barrier layer, which is coated with an adhesive hydrogel. The adhesive layer is covered with backing paper until use to maintain the adhesiveness, and the barrier layer is covered with a protective liner until use to maintain a sterile environment.

Methods Clinical trials demonstrated that not only does the prototype developed perform successfully in terms of the functional properties such as ease of use, barrier properties but also the effect on the intra ocular pressure measurements are acceptably accurate in comparison to the Goldman Tonometer.

Results This poster summarises the work completed to date on the development of the novel sterile universal barrier system and the next stages of further design refinement, involving extended clinical consultation in conjunction with professional design input, interlinking the three aspects: materials, fabrication and ease of use in the clinical environment. In this way, an optimized product with demonstrated acceptability to clinical practitioners can be developed.

Conclusion There is a need for an effective disposable ophthalmic barrier system which is clinically acceptable.

■ 362

Age-related changes in the corneal thickness profile assessed with Orbscan

JONUSCHEITS, DOUGHTY MJ, BUTTON NF

Department of Vision Sciences, Glasgow

Purpose To assess the corneal thickness with scanning-slit (Orbscan) pachymetry at central, mid-peripheral, and peripheral sites and to generate a ratio to describe the corneal thickness profile from the centre towards the periphery as a function of age.

Methods Orbscan measurements were performed on 98 right eyes of 98 healthy subjects. Three readings were taken and data was extracted from the pachymetry maps at the geometrical centre, mid-peripheral locations 2.5 mm to either side of the centre, and peripheral locations 4.5 mm from the centre along the horizontal meridian. Nasal and temporal measurements were averaged for each cornea.

Results The mean age of the subjects was 44.9 +/- 14.1 years (+/- SD), range 19 to 82 years. The mean central corneal thickness was 0.584 +/- 0.041 mm. For the mid-periphery and the periphery the readings were 0.635 +/- 0.042 mm and 0.713 +/- 0.047 mm respectively. The mean M/C-ratio (ratio between mid-peripheral and central corneal thickness) was 1.09 +/- 0.03 and the mean P/C-ratio (ratio between peripheral and central corneal thickness) 1.22 +/- 0.06. The M/C-ratio was only weakly correlated to age. However, the P/C-ratio showed a much stronger correlation to age. The results of our study strongly indicate that corneal thinning does occur at peripheral sites but is not as pronounced in the mid-periphery about 2.5 mm from the centre.

Conclusion Age-related changes in the corneal thickness profile (peripheral corneal thinning) predominantly occur at locations outside 2.5 mm from the centre of the cornea.

■ 363

**Short-term Effects of Contact Lens Corneal Refractive
The Short-term Effects of Contact Lens Corneal Refractive
Therapy on Corneal Topography**

QUEIROS A (1), GONZALEZ-MEIJOME JM (1), VILLA-COLLAR C (2),
JORGE J (1), ALMEIDA JB (1), PARAFITA MA (3)

(1) Physics (Optometry) - University of Minho, Braga

(2) Clínica Oftalmológica Novovisión, Madrid

(3) Surgery (Ophthalmology) - University of Santiago de Compostela, Santiago de Compostela

Purpose The goal of the present study was to investigate central corneal pachymetric changes within the first 3 hours of lens wear under open-eye conditions and the regression of the effect achieved during the same period time after lens removal.

Methods Fourteen volunteers where fitted with Paragon CRT® rigid gas permeable contact lenses according to the fitting recommendations of the manufacturer simulating a different treatment effect of -2 and -4 myopic correction in the right and left eye in random order. After the lenses were placed in both eyes, subsequent measurements were obtained at 30, 60 and 180 minutes, and with same intervals after lens removal.

Results Change in apical curvature above -0.50 D is already detected after 30 minutes of lens wear. However, on average changes in apical curvature are greater (-0.27 vs -0.39 D/hr; p<0.05) and faster (-0.53 vs -0.78 D/hr; p<0.05) for the -4.00 targeted eye. Recovery was total 180 minutes after lens removal. Flat Sim'k showed differences between both treatments even within the first 30 minutes, reaching a maximum reduction in power after 180 minutes of -0.67 and -0.82 D for -2 and -4 targets, respectively (p<0.05). Changes in steep Sim'k were almost the same for both targets (maximum change at 180 minutes: -0.56 vs -0.63 D; p>0.05). Changes in corneal eccentricity were similar for both groups (maximum of -0.17 and -0.19 at 180 minutes of lens wear; p>0.05) with a parallel progression over time.

Conclusion Short term changes in apical radius in response to CRT lens wear are very fast, and apical radius can reach total recovery after short periods of lens wear within 3 hours. From the clinical point of view, this work helps us to understand what to expect from the cornea while we are performing the fitting tests.

■ 364

Gene therapy promotes corneal graft survival

BARCIA RN, DANA RM, KAZLAUSKAS A

Schepens Eye Research Institute, Harvard Medical School, Boston

Purpose Corneal endothelial cells (CEC) are essential to keep the cornea clear. Loss of CEC is thought to occur in graft failure, particularly in graft failure due to rejection, an immune reaction that targets endothelial cells. We postulate, that CEC loss during graft failure is due to apoptosis. Furthermore, because CEC in vivo are thought to have little regenerative capability, we hypothesize that preventing apoptosis in the donor corneal endothelium will promote cornea graft survival.

Methods Anti-apoptotic genes (Bcl-xL, Bcl-2, p35 and survivin) were cloned into a retroviral plasmid vector. Retroviruses were used to infect CEC. Apoptosis was induced by etoposide or IFN γ and TNF α , and detected by annexin V and Propidium Iodide staining and flow cytometry analysis. For in vivo studies, we used an orthotopic cornea transplant model. BALB/c mice were used as recipients, and C57BL/6 or BALB/c (syngeneic) corneas were used as donors. For transduction of the endothelium, excised corneas were treated with eGFP, or IZsGreen or IZsGreen-Bcl-xL lentivirus. Apoptosis in the graft's endothelium was detected by TUNEL staining and confocal microscopy.

Results Apoptosis of the graft endothelium occurred in rejecting corneas as early as 2 weeks. We found that Bcl-xL, but not other genes, protects CEC from apoptosis. Lentiviral-delivery of Bcl-xL to the corneal endothelium of donor corneas significantly improved the survival of low risk allografts.

Conclusion Graft failure is accompanied by apoptosis of the endothelium. Bcl-xL protects CEC from apoptosis in vitro and promotes allograft survival.

■ 365

Influence of corneal factors on tear film in postlasik patients

NIETO A (1), PALOMO-ALVAREZ C (2), CARBALLO-ALVAREZ J (2),
PIELL MC (2)

(1) Optica II, Optometría y Vision, Madrid
(2) Optica II, Madrid

Purpose To evaluate the influence of the corneal asphericity, the ablation and the hinge location in the sintomatología of dry eye and the secretion and stability of tear film in postlasik patients.

Methods Retrospective study of 25 subjects (50 eyes) coming from three clinics of refractive surgery with previous myopia to lasik inferior to 7 D and astigmatism up to 2 D. Patients completed a Mc Monnies questionnaire that evaluated the character and severity ocular symptoms (normal eye, marginal dry eye and dry eye). Tear secretion by phenol red test (PRT, group 1 < 11 mm, group > 11mm), tear stability by fluoresceine breakup time (FBUT, group 1 < 10 s and group 2 > 10s) were measured in each eye. Corneal asphericity was evaluated with Zeis Humphrey Systems Atlas. The pre and post corneal thickness and ablation were collected from clinics data base. The data were analyzed with t-Student and chi-square.

Results The results showed that the average values of corneal asphericity were not different in the groups FBUT, RPT and Mc Monnies scores. Depth ablation was significantly higher in patients with BUT and score of MC Monnies within normality. The percentage of women with a tear secretion below the normal levels was significantly higher than men group ($p= 0.0039$). The percentage of eyes with superior hinge that presented a BUT below the normal limits was significantly greater than the percentage with nasal hinge ($p= 0.004$).

Conclusion Lower FBUT was found in patients with lower depth ablation. Lacrimal secretion was minor in postlasik women group and worse lacrimal stability was found in patients with superior hinge.

■ 366

A new system for grading staining at the surface of the eye

BRON AJ (1), LANG C (2), FOULKES GN (3), SMITH JA (4), TIFFANY JM (1)

(1) Nuffield Laboratory of Ophthalmology, Oxford
(2) Dept. of Materials, Oxford
(3) Dept. of Ophthalmology, Louisville
(4) National Eye Institute, Bethesda

Purpose To create a standardised system for grading staining at the ocular surface using a mathematically-defined series of panels.

Methods Three current systems compare staining with panels of dots of increasing number density. Scale ranges vary from 0-9 (van Bijsterveld), 0-15 (Oxford) and 0-33 (NEI/Industry workshop). The latter system grades over multiple corneal zones, so that different phenotypes may be described numerically and the effect of central staining on visual function may be addressed. We have modified the NEI/Industry system: i. to standardise size and location of recording zones and ii. to create panels of random dots whose increasing number density from panel to panel is mathematically defined. To do this: 1. a series of 10 panels was generated, with the probability p of a pixel being black given by: $p = \exp(0.9^a)/\exp(0.9^b)k$, where: a = number of the current panel; b = number of the last panel + 1; k = 0.00042. In order that dot size on the printed panels would approximate to that of a staining point on the ocular surface, 2. the panels were scaled in MSWord so that dot size would reflect the average size of an epithelial cell and the magnification of the slit-lamp used.

Results Here we illustrate how such panels may be constructed and modified to reflect clinically encountered staining densities. A standard recording template is presented and a set of panels providing a grading range of 0-10 for each zone assessed. The scale range of the system is 0-110.

Conclusion The utility of the system presented is under review.

■ 367

Experimental model of corneal wound healing after implantation of intracorneal rings and segments (ferrara)

BLANCO-MEZQUITA JT (1), MERAYO-LLOVES JM (1), FABIANI L (1),
BARCIA A (2), FERRARA P (3)

(1) Instituto Universitario de Oftalmobiología Aplicada, Valladolid
(2) Animal Facilities, Valladolid
(3) Advanced Center of Ophthalmology, Bello Horizonte

Purpose Intracorneal rings and segments are an alternative of surgical treatment for keratoconus, corneal ectasia and eventually avoid keratoplasty. This segments could be used for ametropia correction. The purpose of this research is to evaluate the effectiveness of intracorneal application of intrastromal ring segments, both in corneal wound healing process and corneal transparency.

Methods PMMA segments were implanted in 24 Iber braun hens corneal model. Clinical follow-up was carried under a surgical microscope. At different time points animals were euthanized and the corneas were fixed in 10 % buffered formaline. Morphologic changes were evaluated by optic (H-E) microscopy examination. Apoptosis and proliferation of keratocytes and myofibroblast differentiation were evaluated.

Results A few apoptotic keratocytes was found near the injury zone. The number of apoptotic cells was higher in the corneas of 12 hours than 24 and 72 hours. Proliferation of keratocytes was found around the injured zone and nearby the corneal limbus at 72 hours. Myofibroblast were found around the injured zone. The area of myofibroblast localization was higher in the corneas of 15 hours than 30 days. Two months after surgery myofibroblasts were not found.

Conclusion An animal model of corneal wound healing after ICRS implantation was developed. In this animal model, PMMA segments were well tolerated with a minimal histopathological response.

■ 368

Design of a new keratoprosthesis: evaluation of the colonization of polymeric biocompatible scaffolds

PASTORS (1, 2), ALIO JL (3, 2), RUIZ-MORENO JM (3, 2),
CAMPILLO-FERNANDEZ A (4), GOMEZ-RIBELLES JL (4, 5),
MESEGUEIR-DUENAS JM (4)

(1) Research, Development and Innovation, Instituto Oftalmológico de Alicante (VISSUM), Alicante
(2) Universidad Miguel Hernández, Alicante
(3) Ophthalmology, Instituto Oftalmológico de Alicante, Alicante
(4) Biomaterials, Centro de Biomateriales, Universidad Politécnica de Valencia, Valencia
(5) Centro de Investigación Príncipe Felipe, Valencia

Purpose To analyze the biocolonization properties of different porous materials with different compositions of polymeric acrylates and methacrylates to find an ideal material to be used as the scaffold of a future keratoprosthesis.

Methods Adipose-Derived Adult Stem Cells (ADASC) and a cell line of keratocytes were cultured on different substrates with different content of hydrophilic/hydrophobic monomers made of acrylates and methacrylates (pEA and pHFA) at different ratios. Porous materials made of 100% pEA were also evaluated. 5.104 cells per biomaterial in triplicates (1cm diameter and 1mm thick) were grown and their adhesion and proliferation rates analyzed by optical microscopy, SEM and Trypan Blue staining. Cells were evaluated on days 0, 3, 5 and 7.

Results Proliferation of ADASC and keratocytes was observed on materials with a main component of the hydrophobic poly ethylacrylate (pEA). The expansion and colonization of these cells on 100% pEA porous materials was only observed on the surface. Large collagen deposits and producer cells covered the surface and no penetration was observed by SEM.

Conclusion Hydrophobic 100% pEA showed to be very efficient on adhesion and proliferation of ADASC and keratocytes on bi-dimensional substrates. Porous materials made of 100% of this polymer did not show colonization on the inside of the channels probably due to the hydrophobic nature of this material. Future substrate will combine other hydrophilic materials to make the scaffolds more biocolonizable.

■ 369

Safety of Mitomycin c (MMC) in reepithelization after LASEK

PIZZAMIGLIO-MARTIN C (1), DE BENITO L (2), FUENTES I (3)

ALVAREZ MT (4), TEUS MA (2, 5)

(1) Cornea and ocular surface, Vissum-Hospital Oftalmológico, Madrid

(2) Cornea and refractive surgery, Vissum-Hospital Oftalmológico, Madrid

(3) Optometry, Vissum-Hospital Oftalmológico, Madrid

(4) Retina, Vissum-Hospital Oftalmológico, Madrid

(5) University Hospital, Alcalá

Purpose Mitomycin C (MMC) is useful to avoid haze after superficial ablation, but it has shown toxicity in other surgeries. Our purpose is to study the effect of using MMC in corneal reepithelialization after LASEK.

Methods We performed a retrospective study of consecutive patients operated with the LASEK technique by the same experienced surgeon. We excluded patients with local or systemic disease that could have interfered in reepithelialization. We separated two groups age-matched: in group 1, those whose ablation depth was >50 microns, who received MMC during 30sec over the ablated zone; in group 2, those whose ablation depth was ≤50 microns, who received no MMC. In both groups a soft contact lens was placed. Postoperative visits were performed by a masked observer on days 1, 5, 30 and 90 after surgery. We compared the number of extra visits that were necessary to assure complete reepithelialization after day 5 in both groups.

Results This pilot study includes 103 eyes (60 in group 1; 43 in group 2). The number of additional visits was 0,3±0,6 in group 1 and 0,2±0,5 in group 2 ($p=0,09$). No trophic defect was found later than 10 days after surgery in either group. No other complications were reported.

Conclusion The use of MMC seems not to cause a delay in reepithelialization after LASEK.

■ 370

The Persistent Enemy - recurrence of herpes simplex in three consecutive corneal graft buttons

HUNTBACH JA (1, 2), WORSTMANN T (2)

(1) Queens Medical Centre, Nottingham

(2) Queens Hospital, Burton-on-Trent

Purpose To demonstrate that, despite effective medication, herpes simplex infection in the remaining corneal rim can persist and reinfect successive corneal buttons following penetrating keratoplasty (PK).

Methods A 69 year old woman presented at Queens Hospital, Burton in 1980 with a 12 year history of recurrent herpes simplex keratitis (HSK). She also suffered from Sjogrens syndrome. In 1988 she developed a pseudomonas ulcer which necessitated her first PK. She had recurrence of HSK in 1991 and, despite appropriate therapy, several further episodes over the next two years. In 1993 she developed graft rejection which progressed to a descemetocèle and required a second PK. She later developed corneal melting at the graft/recipient interface and required immune suppression. Over the next seven years there were several more recurrences of HSK and a corneal melt in 2004 resulted in a third PK. Eight months later, she underwent amniotic membrane grafting for graft leakage. A further 3 months later she developed endophthalmitis and underwent evisceration.

Results Histopathology reports -28.4.89 "Vascularised corneal disc containing a healed ulcer with scarring." 18.8.93 "A degree of active HSV keratitis." 25.2.04 "Active HSK occurring in relation to the host-graft junction." 14.1.05 "Features of an ongoing keratitis in keeping with previous herpes simplex infection."

Conclusion Herpes simplex infection recurred in every corneal button following initial grafting, demonstrating how difficult it is to prevent the virus crossing the graft/host interface to infect fresh graft tissue. This case supports the use of high dose oral acyclovir as prophylaxis for graft recipients with previous HSK as suggested by the "Herpetic Eye Disease Studies"

■ 371

Which is more difficult:**The test to use or the diagnosis of Dry Eye?**

MOORE JE (1), GRAHAM JE (2), DARTT D (3), GOODALL E (2), MOORE CBT (2)

(1) RVH, Belfast

(2) UU, Coleraine

(3) Schepens Eye Research Institute, Boston

Purpose Diagnosis of dry eye contains a myriad of different conditions and variants of one condition. This use of deterministic diagnostic tests produces unexpected and potentially contradictory results. Most dry eye studies account for sensitivity and specificity problems by taking 2 out of 3 positive results as a positive indication of dry eye status. We sought to combine probabilistic and deterministic techniques to produce an appropriate diagnostic algorithm for dry eye diagnosis.

Methods A consultant ophthalmologist performed a combination of objective and subjective clinical examinations on 91 subjects. These included PRT, TBUT, questionnaire, impression cytology for goblet cell grading and biomicroscopy. Inter test correlation was sought using a probabilistic statistical approach (McNemars test). All tests showing no inter test correlation were ignored and a scoring system was generated weighted to the relevant test results.

Results Only 4 specific tests demonstrated correlation: McMonnies questionnaire, FTBUT (cut off 7 sec), goblet cell grading and MGD severity grading (p value = ns). PRT, Lid erythema, Lid swelling, conjunctival erythema & tear film debris all showed no correlation (p value <0.001).

Conclusion A diagnostic algorithm was devised using correlated tests each weighted to account for provisional results and clinical test value. This algorithm resulted in 34 patients diagnosed as dry eye and 57 normal, a change in diagnosis of 6 subjects compared to the diagnostic mechanism of any 2 combined positive clinical tests. No benchmarks are available to determine if this is an appropriate method to determine dry eye status, however it may be a valuable approach to recognise which tests are relevant to the patient base under investigation.

■ 372

Anti-BP180 antibodies in Ocular Pemphigoid

ROBERT PY (1), AL YOUSSEFA (2), BEDANE C (3), COGNE M (2), ADENIS JP (1), DROUET M (2)

(1) Ophthalmology, Limoges

(2) Immunology, Limoges

(3) Dermatology, Limoges

Purpose BP180 is a molecule constitutive of hemidesmosomes and an important target of antibody response in ocular cicatricial pemphigoid (OCP) and other bullous disease (BD). Anti-BP180 antibodies can recognize different part of the molecules. Our observation, based on microscopy imaging, suggested that an epitope could be specific of the OCP. Therefore we investigated this hypothesis with recombinant protein.

Methods Two recombinant peptides were designed using pGEX plasmid expressed in E. Coli: the COOH protein is localized at the extremity of the extra cellular section of BP180 whereas the NH2 protein encompass the trans- and juxta membrane section. These peptides were used to setup two ELISA test. The reactivity of serum of OCP patients (N=40) were compared to the one of bullous pemphigoid (83 patients).

Results The NH2 protein tested was a target of the immune response respectively for 50% of OCP patients and 68.8% of the BD patients. The COOH protein was a target in 9% of the OCP group and 25% of the BD group. The differences are not significant.

Conclusion The trans and juxta membrane region of BP180 protein is the major target of the antibody response. None of those proteins reveal to be a specific target of OCP. Nevertheless, these Elisa tests, based on recombinant protein, prove to be useful tool for the diagnosis as well as the analysis of the physiopathology of the disease.

■ 373

Automatic estimation of corneal endothelial cell density in images from swollen organ cultured donor corneas

RUGGERI A (1), GRISAN E (1), SCHROETER J (2)

(1) University of Padova, Padova

(2) Cornea Bank Berlin, Charité, Berlin

Purpose Estimation of density of corneal endothelial cells with an available software system in images from swollen donor corneas was investigated.

Methods We developed an algorithm for the automatic estimation of endothelial cells density (ECD) in images acquired at eye banks prior to organ culture or after de-swelling (Ruggeri et al., Br J Ophthalmol, 89, 2005). It is based on the frequency analysis of the pattern visible in the images, from which information leading to ECD can be derived. The algorithm is also capable of assessing when the quality of the acquired image is not adequate to derive a correct estimation of ECD, so as to discard the image. In order to assess its performances when corneas are examined in swollen conditions, images were acquired in 41 such corneas and analyzed with the module. Reference manual counts were performed on each image by an experienced ophthalmologist.

Results The software discarded as non-processable images from 30 corneas. In the remaining 11, the average difference of automatic densities vs. manual ones was -55 cells/mm² (-2%), with std dev of 72 cells/mm² (3%) and range -140 ± +72 cells/mm² (-7% ± +3%). Average absolute difference was 76 cells/mm² (3%), with std dev of 47 cells/mm² (2%) and range 2 ± 160 cells/mm² (0% ± 7%). Running times were in the order of 1 second per image.

Conclusion On swollen corneas, the software discarded many more images than on de-swollen corneas, where acceptance rate is approx. 86% (unpublished results). On analyzed images, however, ECD was estimated with very good accuracy. If multiple images per cornea were acquired in the swollen conditions at different focal depth, the percent of accepted images should markedly increase.

■ 374 / 2416

Mutation analysis of RHO gene and novel mutation Arg252Pro (755G→C) in patients with nonsyndromic retinitis pigmentosa from Bashkortostan

GRINBERG E, DZHEMILEVA L, KHUSNUTDINOVA E

Institute of biochemistry and genetics, Ufa

Purpose To identify mutations in the RHO gene in patients with nonsyndromic retinitis pigmentosa (RP) from Bashkortostan. RP is a clinically and genetically heterogeneous group of retinal degenerative diseases. The prevalence of RP is 19 to 27 per 100000 in general population. In the adRP approximately 30% of families have mutations in the RHO gene.

Methods We examined all exons of RHO gene in 120 unrelated patients with RP and 120 unaffected individuals by single-strand conformation polymorphism analysis (SSCP) and direct sequencing. Patients were examined clinically and with visual function tests.

Results When the entire RHO gene was examined, we detected sequence change IVS3+4c→t, and its frequency more in patients (0.36) than in controls (0.1). Also we analyzed polymorphism Mfd2CA of RHO gene and revealed 14 allelic variants. Also we report the identification of a novel mutation Arg252Pro (755G→C) of the 4 exon RHO gene.

Conclusion There were statistically significant differences in allele and genotype frequencies of polymorphism IVS3+4c→t of RHO gene in affected patients with RP and in controls. So, according to our data, this polymorphism is likely to be pathogenic. Alleles 116 and 132 of polymorphism Mfd2CA of RHO gene are significantly more frequent in patients than in controls. Novel mutation Arg252Pro (755G→C) of the 4 exon RHO gene was detected in 1 bashkirian family with RP. All family members carried mutation Arg252Pro in heterozygous form and have similar fundus appearances. Identification of the molecular defects underlying retinal degeneration will allow clinicians to establish more accurate diagnoses and prospects for prenatal diagnostic.

■ 375 / 2417

Familial high myopia in Polish population

RYDZANICZ MA (1), PODFIGURNA-MUSIALAK M (2), FRAJDENBERG A (3), BEJJANI BA (4), LEAL SM (5), GAJECKA M (1, 4)

(1) Institute of Human Genetics Polish Academy of Sciences, Poznan

(2) Department of Ophthalmology, Hospital, Leszno

(3) Department of Ophthalmology, Marcinkowski University of Medical Sciences, Poznan

(4) Washington State University, Spokane

(5) Baylor College of Medicine, Houston

Purpose Myopia is the most common of all ocular conditions. Although high myopia (myopia in excess of - 6.0 diopters [D]) is far rarer than mild/moderate myopia, the importance of high myopia is significant because the development of high myopia involves anterior-posterior enlargement of the eye, abnormal changes in the eye and frequent detachment of the retina. The etiology of myopia is not known. Both genetic and environmental factors seem to play a role. The aim of our study is mapping and cloning gene(s) responsible for familial high myopia in Polish population.

Methods We have examined collected blood and purified DNA from 199 individuals from 32 unrelated high myopia families in Poland. As a first step before embarking on a genome-wide screen the preliminary genotyping was conducted in 23 families. Prior to preceding with the targeting genotyping, linkage to markers for Stickler syndrome types I, II and III; Marfan syndrome and juvenile glaucoma were tested. Next, we have examined previously proposed familial high myopia loci [18p11.31 (MYP2), 12q21-23 (MYP3), 7q36 (MYP4), 17q21-23 (MYP5), 2q37, 4q22-4q27 (MYP11), and 10q21.1], and additionally to test the linkage for mild/moderate myopia established in Ashkenazi Jewish families and dizygotic twins, 22q12 (MYP6) and 11p13 (MYP7) were genotyping as well.

Results Linkage to Stickler syndrome, Marfan syndrome and juvenile glaucoma loci were excluded. Genotyping with well-spaced polymorphic markers of high myopia associated loci revealed no evidence of linkage to any of the candidate genes.

Conclusion There was no evidence of linkage to any of candidate genes and high myopia loci. Further genetic studies are required, genome-wide screen is in progress.

■ 376 / 3216

Delayed development of Retinal Vessel Network in Alpha 2B Adrenergic Receptor Knock-out Mice

HUA J (1), MUTHIG V (2), GROSS N (1), LANGE CAK (1), MARTIN G (1), HANSEN LL (1), HEIN L (2), AGOSTINI HT (1)

(1) University Eye Hospital, Freiburg

(2) Institute of Experimental and Clinical Pharmacology and Toxicology, University of Freiburg, Freiburg

Purpose Alpha 2 adrenergic receptors (Adra2) mediate diverse biological effects of the endogenous catecholamines epinephrine and norepinephrine. Subtype Adra2b was found to play an essential role for placental vascular development, whereas the other two subtypes Adra2a and c are presynaptic feedback regulators of sympathetic nerves. To determine its role in vasculogenesis and angiogenesis, we studied the development of retinal vasculature in Adra2b knock-out (Adra2b-/-) and wild type C57Bl/6 mice.

Methods Adra2b-/- and C57Bl/6 mice underwent intraventricular perfusion with FITC-dextran solution. After enucleation, retinal flat mounts were prepared at different time points: first (p1), fourth (p4), seventh (p7) and tenth (p10) postnatal day (n=4 per time point and genotype). The ratio of vascularised area to total retina was taken as an index for completed vascularisation.

Results There is significant delay of vascularisation in Adra2b-/- mice on p4 and p7 compared to the control group. Both in Adra2b-/- mice and C57Bl/6 mice about 5% of the central retina is vascularised on p1. The vessel network reaches the edge of the peripheral retina on p10 in both genotypes.

Conclusion The development of the murine retinal vasculature is influenced by Adra2b. However, the cellular mechanisms involved remain unknown. Recent studies indicated that activation of the mitogen-activated protein kinase (MAP kinase) pathway by Adra2 is required for placenta and yolk-sac vascular development. Future studies will have to address whether the findings derived from knockout mice are relevant for human physiology and retinal disease.

■ 377 / 3217

Temporal expression profile of murine cornea: a possible role for Vsx1 in corneal development?

SHEPPARD JPB (1), MEEK KM (1), VOTRUBA M (1, 2)

(1) Optometry and Vision Sciences, Cardiff

(2) Cardiff Eye Unit, University Hospital Wales, Cardiff

Purpose To examine the expression of a number of corneal genes, including the paired-like homeobox gene Vsx1 in postnatal mice during eye lid opening in order to study the role of these genes in corneal development.

Methods Whole murine eyes, brain, liver, lung and heart from 4 mice were extracted and placed in RNAlater at P5, 12 and 17 of development. Eight corneas at each time point were dissected out and extracted RNA was quantified and transcribed to cDNA for quantitative PCR. cDNA was amplified with SYBR Green for real-time quantitative PCR using RNA specific primers for the following genes: Vsx1, Aqp1, Aqp5, and Col8A2. Rpl19 was used as a housekeeping gene for normalization.

Results Vsx1 shows a ~2 fold increase in expression from P12 to P17 in the cornea, while expression was absent at P5. Vsx1 expression was also found in the brain and liver at P12 and in the brain, liver and lung at P17. Expression of Aqp5 has a ~2.50 fold higher expression and Aqp1 has a ~3.8 fold increase in expression from P12 to P17. Col8A2 shows highest expression at P5 with a subsequent 2.4 fold decrease at P12 and 2.83 fold decrease at P17.

Conclusion The period of eyelid opening in murine development sees many changes especially in corneal thickness. Mutations in Vsx1 have been implicated in keratoconus, a corneal thinning ectasia and the gene Col8A2 has been linked with PPCD. Our data indicate that expression of the aquaporin genes examined increases from P12 to P17. Of note is that Vsx1 expression is also increased from P12 to P17 while expression of Col8A2 decreases at the same time points. As both these genes have been implicated in PPCD there is the possibility that these two genes interact, which could merit future study.

■ 378

Ocular manifestations of Nail Patella Syndrome

RAJA V (1, 2), SANDANSHIV P (3), NEUGEBAUER M (3), SWEENEY E (4)

MCINTOSH I (5)

(1) The University of Liverpool, Liverpool

(2) The Eye Centre, Leighton Hospital, Crewe

(3) The Eye Care Centre, Leighton Hospital, Crewe

(4) Department of Genetics, Royal Liverpool Children's Hospital, Liverpool

(5) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore

Purpose To report ocular abnormalities in patients with Nail Patella Syndrome (NPS).

Methods In an observational case series study, we studied the eyes of 7 patients with genetically confirmed NPS. Examination included slit lamp bio-microscopy of the anterior segment and fundus, applanation tonometry and gonioscopy. Digital photographs of the anterior segment and the anterior chamber angle were taken and studied.

Results In this initial study, all our patients were noted to have anterior chamber angle abnormalities which comprised of prominent strands of iris tissue attached to the trabecular meshwork resembling an Axenfeld's type of anomaly. Open angles were seen in all our subjects. Cloverleaf pattern of the central iris (Lester sign) was noted in 3 patients. 4 patients were known glaucoma patients and were already on treatment.

Conclusion We speculate whether NPS belongs to the spectrum of anterior chamber cleavage syndromes as a variation of Axenfeld's type anomaly has been clearly demonstrated in the anterior chamber angle. Lester sign, though not pathognomonic, occurs more frequently in patients with NPS as demonstrated in a British study. Glaucoma is already a known association of NPS. Genetic analysis of these patients showed an abnormality in the LMX1B gene on chromosome 9q34. LMX1B gene has been shown to be expressed in the anterior chamber of the developing eye in humans and mouse models. Further studies with a larger group of patients with NPS are needed to prove whether the angle abnormalities as demonstrated in our study actually contribute to the development of glaucoma.

■ 379

Management and visual outcome in children with Lowe syndrome

PINELLO L (1, 2), MAIMONE PE (1, 2), MAZZAROLO M (1, 2), CALDIRONI P (3)

(1) Paediatrics, Padua

(2) Paediatric Low Vision Centre, Padua

(3) Hollman Foundation, Padua

Purpose Lowe syndrome is a rare (1:500,000), X-linked, disease characterised by anomalies involving the eye (cataract, glaucoma), nervous system (hypotonia, mental retardation) and kidneys (Fanconi's tubulopathy). We evaluated the long-term visual outcome and the complications in 4 children affected by Lowe.

Methods Four male children, mean age 96±46 (36-146) mths, underwent cataract surgery at a mean age of 4±2.94 (1-8) mths. The follow-up ranged from 31 to 127 mths (57.8±43.5). Complete ophthalmologic evaluations were performed at regular intervals. Visual acuity and intraocular pressure (IOP) were assessed by Preferential Looking Technique and TonoPen XL respectively. Optical correction (contact lenses, glasses) was prescribed upon hand-held automatic refractometry. Children underwent a complete and integrated rehabilitative pathway, in order to improve visual skills and thus the neurocognitive development.

Results Visual Acuity was 5.41±3.56 (0.32-13) cy°/m of grating acuity corresponding about to 2/10 in Snellen fraction. IOP rose up in 2 patient; at first evaluation IOP was 28 and 36 mmHg respectively, and only in one eye. After treatment with topical beta-blocker and/or prostaglandin agonists, at last evaluation IOP was 20 and 15 mmHg.

Conclusion The management of ocular disorders of Lowe syndrome includes, first, the early detection and surgical approach of cataract. After this first stage, children should be followed-up at regular intervals to assess the visual function, the IOP, the refractive variations and the other possible ocular complications. Only a careful approach from the first diagnosis, despite the poor cooperation due to mental retardation, can optimize the long term visual outcome in children affected by Lowe syndrome.

■ 380

VMD2 mutations in Italian patients with Best maculopathy

SODI A (1), PASSERINI I (2), BINI A (1), MENCHINI U (1), TORRICELLI F (2)

(1) Department of Ophthalmology, Careggi University Hospital, Florence

(2) Department of Genetic diagnosis, Careggi University Hospital, Florence

Purpose Vitelliform macular dystrophy (Best's disease) is an autosomal dominant, early-onset form of macular degeneration . VMD2 gene, considered responsible for the disease, mapped to the long arm (q13) of chromosome 11, and it codifies a protein (BESTrophin) which probably acts as a chloride channel. In the present study we screened for mutations the VMD2 gene in Italian patients with Best maculopathy.

Methods Seven families with Best disease were recruited from central Italy. All affected patients and some of the unaffected family members had a blood sample drawn, and the DNA was analyzed for mutations in all 11 exons of the VMD2 gene by DHPLC approach (with optimization of DHPLC conditions for mutation analysis) and direct sequencing techniques.

Results Some mutations of the VMD2 gene have been detected in all the affected patients and in some unaffected relatives. Most of the mutations have already been described in the literature; a novel mutation (R218G) was detected. It is a missense mutation and it involves an aminoacid which has been reported to be highly conserved across different species. Sometimes the same mutation was associated with some differences in clinical phenotypes. In one family the R218C mutation was associated with the onset of choroidal neovascularization (CNV) in the affected mother and her son, while no CNV was reported in another family sharing the same mutation.

Conclusion Some mutations of VMD2 gene have been detected in some Best's disease families; among them there is a novel mutation probably involving a functionally active region of the bestrophin protein. In spite of the small number of studied families it was possible to remark a certain phenotype heterogeneity

■ 381

Clinical and genetic study of myopia in St. Peter island (Sardinia)

ZUCCA I (1), GALANTUOMO MS (1), MALLOCIC (1), STAMBOLIAN D (2)
FOSSARELLO M (1)

(1) Eye Clinic, University of Cagliari, Cagliari
(2) Ophthalmology University of Pennsylvania, Philadelphia

Purpose To identify and collect pedigrees affected with high myopia from an homogeneous ethnic group (San Peter island population, Sardinia).

Methods Families with at least one member affected by high myopia (6 diopters or more) since early childhood have been screened clinically (subjective and objective refraction, slit lamp, US biometry, corneal topography, fundoscopy) in order to evaluate the distribution and pattern of inheritance of the trait. Blood samples from suitable families have been collected for DNA extraction and DNA genotyping.

Results We identified 15 families in which high myopia is transmitted as autosomal dominant trait. Two main phenotypes have been observed: a pure spherical myopic defect, and an compound myopic astigmatic defect. Unilateral cases have also been included.

Conclusion Preliminary DNA examination demonstrated close linkage between D18S63 marker and high myopia. Our results provide additional evidence that high myopia is sustained by a genetic background.

■ 382

Follow-up of young patients with retinitis pigmentosa by multifocal ERG

VAMOS R, LESCH B, SOMFAI GM, FARKAS A

Semmelweis University, Department of Ophthalmology, Budapest

Purpose To evaluate electrophysiological changes in young patients with retinitis pigmentosa.

Methods 15 eyes of 8 patients aged 8-19 years (mean 13) underwent electrophysiological follow-up by multifocal ERG (RETI-scan, Roland-Consult, first order kernel, 61 hexagons, 5 concentric rings) for a period of 1-5 years (mean 2, 8). Statistical analysis was carried out by Wilcoxon paired test.

Results There were no significant differences in response density values of the b-wave between first and control examination in children under the age of 10 years (4 patients, 7 eyes). Alterations in response density values showed significant decrease in the third /5-13°/ ($p=0,02$) and fifth /17-30°/ ($p=0,04$) rings between first and control examinations in patients aged 10-20 (4 patients, 8 eyes).

Conclusion Worsening of retinal function seems to be more progressive in teenager patients with early-onset retinitis pigmentosa than in children under the age of 10.

■ 383

Visual and ocular motor functions in Russian orphanage children with fetal alcohol syndrome (FAS)

GUMMEL KK (1, 2), YGGE J (2), BRZHESKY VV (1)
(1) Ophthalmology, St-Petersburg State Pediatric Medical Academy, St-Petersburg
(2) Clinical Neuroscience, Stockholm

Purpose Despite long studying of FAS still there is a lack of knowledge about this syndrome and co-existing ophthalmological problems in Russia. We've studied visual and ocular motor functions in a group of Russian orphanage children with FAS.

Methods 100 children of 10-16 years of age were examined: 50 - with verified diagnosis of FAS and 50 healthy children. To all children were carried out visorefractometry, skiascopy, dynamic and central static computer (RBP) perimetry, colorimetry, and also functions of tracking (Clinsacc and Clintrac tests) were studied.

Results FAS children differ from control children in that they have a lower visual acuity, more refractive errors, more accommodative problems, higher incidence of strabismus, ptosis and microphthalmos, lower hit rate in RBP and poorer tracking ability. The results from the present study shows that a large proportion of Russian FAS children have a lower visuo-motor performance compared to non-FAS children.

Conclusion Results of examination testify to disordered eye's functions at children with FAS. This finding urges for a thorough ophthalmological investigation in such children and must be taken into consideration when planning for their future

■ 384

Palpebral fissure size of aged people in Korea

JEON SL, PARK CY, WOO KI, CHANG HR
Ophthalmology, Seoul

Purpose We analyzed palpebral fissure size in normal senior citizens, and we wanted to find out how many of them felt discomfort from droopy eyelid.

Methods In this study, 754 eyes of 377 aged people visiting community centers were included. The average age was 72 years (ranging from 51 to 89). In there 50's were 31(8.3%), 60's were 75(19.8%), 70's were 198(52.7%), and 80's were 73(19.2%). There were 200 men(53%) and 177 women(47%). Margin-reflex distance 1(MRD1) and palpebral fissure height(PF) were evaluated with an attempt to eliminate eyebrow elevation. Levator function(LF) was measured with Berke's method. The examined people fell into two groups, the decompensated group having discomfort from their droopy eyelids and the compensated group not having any.

Results The average of MRD1, PF and LF was 1.6 ± 1.2 mm, 5.7 ± 1.4 mm, and 10.5 ± 2.0 mm, respectively. The values of MRD1, PF, and LF decreased with increase of age, and which were statistically significant($P<0.05$). Compensated group was 344(91.2%) and decompensated group was 33(8.8%). The averages of MRD1, PF, and LF of former were 1.7 ± 1.2 mm, 5.8 ± 1.5 mm, 10.5 ± 2.0 mm, and those of latter were 1.3 ± 1.0 mm, 5.2 ± 1.2 mm, 10.3 ± 1.7 mm. The values of MRD1 and PF between two groups were different ($P=0.001$, 0.000) but those of LF were not ($P=0.256$).

Conclusion The parameters of palpebral fissure size of aged Korean people were much smaller than normal values if elevation of the eyebrow was forbidden. There seems to be a degenerative process in eyelid elevation considering that the palpebral fissure size was getting smaller with increase of age. In most senior citizens, they had no apparent discomfort from droopy eyelid compensating it with eyebrow elevation.

■ 385

Golf-related ocular injuries: injury pattern, visual outcome and prevention

GAWLEY SD, WILLIAMS MA, RANKIN SJA, WILLOUGHBY CE, FRAZER DG
Ophthalmology, Royal Victoria Hospital, Belfast

Purpose Although golf-related ocular injuries account for a low proportion of ocular injuries overall, they can be the most serious. This study examines the nature of such injuries, intervention required, visual outcomes and prevention measures.

Methods Cases were identified and data recorded by retrospective case note review.

Results Six cases were identified. Five of the patients were injured by a golf ball and one with a golf club. All of the patients sustained closed globe injury of varying severity. Two of the patients required surgery – in one case plating of a blow out fracture and in the other trabeculectomy for angle recession glaucoma. Final Snellen visual acuities were 6/5, 6/6 (two cases), 6/36, Counting Fingers and Perception of Light.

Conclusion Golf-related ocular trauma is usually severe. This case series demonstrates the degree of damage which can occur. Even in closed globe injury surgery may be required at a later stage. Methods of prevention include strict adherence to golfing etiquette, the use of protective eyewear is generally unacceptable.

■ 386

Spontaneous iris bleed and management options

SKINNER KC (1, 2), KHETPAL S (1), CHRONIJC (2), MURRAY C (2)

(1) *Reading eye department, Reading*
(2) *Conquest hospital, St-Leonards-on-Sea*

Purpose To highlight an unusual clinical finding in painless blurred vision and discuss possible ways of managing such a case.

Methods Case report

Results A 54 year old gentleman presented on a Saturday to eye casualty with a sudden onset of unilateral painless blurred vision. He had been out shopping at the time. There was no previous history of trauma. No past eye history and he was emmetropic. There was no family history of note. He was fit and well on no medication. When examining the eye it was noted that he had an iris artery bleeding at exactly 12 o'clock in the affected eye. The iris did not look in anyway abnormal, with no prominent vascular tufts. The ocular examination was otherwise entirely normal. Whilst formulating a plan to stop the bleeding, it fortunately spontaneously self resolved. He has had no further problems with the eye and the blood cleared with no sequelae to date.

Conclusion Fortunately in this case the bleeding resolved spontaneously. However this represents an unusual finding and therefore could pose difficulties in its management. This is especially so for a junior doctor in an out of hours situation such as here. We have therefore done a literature search to see what has previously been described. To date there is a paucity of publications regarding this topic. Of the few articles there is, the only described treatment option is that of direct argon laser. To the best of our knowledge there appears to be no similar reported cases. There needs to be encouragement of reporting so as to compile possible treatment options should it occur in your department.

■ 401 / 3127

Early diagnosis of ocular motility alterations in thyroid autoimmune ophthalmopathy (TAO)

VARANO L, CARNOVALE SCALZO G, CAMPAGNA F, SCORCIA GB, ROTONDO E, PENDINO V, PARISE S, BRUZZICHESSI D, PAOLA A, SCORCIA G
Eye Clinic, Magna Graecia, University, Catanzaro

Purpose Object of our study is early diagnosis, through EOMG exam, of ocular motility alterations in subjects affected by TAO without clinical evidences.

Methods 40 eyes of 20 patients affected by TAO without clinical evidences were examined. All the patients were submitted to ocular motility exam and EOMG exam. Results were related with a group of 46 eyes of 23 subjects with same age and sex without ocular motility alterations and not affected by TAO. We used BM6000MOE system and WinEOMG software for signal stimulation and acquisition, WinElabEOMG software for results elaboration.

Results In 15% of observed patients we found an increase of movement speed dissinergy; in 60% of patients we found an increase of movement maximal speed; in 45% we found a decrease of movement medium speed.

Conclusion In TAO affected patients without evident ocular clinical signs, the analysis with EOMG sistem could bring an ocular motility alteration predictive value.

■ 402

Variation of fusional vergences with age and their relation to type of horizontal heterophoria

VIDAL LOPEZ J, SORO MARTINEZ MI, SOBRADO CALVO P, SANCHEZ-MIGALLON MC
Ophthalmology, Murcia

Purpose Fusional vergences (FV) and horizontal heterophorias (HH) reflect different important aspects of the visual function. While the former measure the ability of both eyes to converge or diverge maintaining the fusion and avoiding blur, the latter give us information about the position of the visual axis in absence of fusion stimulus. The aim of this work was to investigate the relation between both parameters.

Methods In a sample of 281 students, 171 boys and 110 girls, the HH and the break and recovery of positive (PFV) and negative fusional vergences (NFV) were registered using a prisms bar. The measurements were made at far and near distance vision, using an appropriate test for each distance.

Results The mean age of the sample was 12.39 ± 1.48 years, in a range from 9.42 to 16.42 years. The correlation coefficients between the HH and the values of break and recovery of the FV, at far and near distance, were obtained. The value of the break of the PFV tended to increase with age, in agreement with the significantly increase in the far distance heterophoria registered in esophoric subjects ($r=0.24$, $p=0.047$); however, the NFV tended to diminish with age, in agreement with the significantly increase in the near distance heterophoria registered in the exophoric subjects (break: $r=0.33$, $p<0.001$; recovery: $r=0.28$, $p<0.001$).

Conclusion The variations of the FV with age are influenced by the type and rank of HH in the population studied. Specifically, the break of the PFV at far distance tend to increase with age in esophoric subjects, meanwhile the break and the recovery of the NFV at near distance tend to decrease with age in exophoric subjects.

■ 403

Variation of the binocular function based on the age

SANCHEZ-MIGALLON MC, VIDAL LOPEZ J, SOBRADO CALVO P, SORO MARTINEZ MI
Ophthalmology, Murcia

Purpose The aim of this work was to investigate if the values of horizontal phoria, near point of convergence (NPC) and positive and negative fusional vergences are related to the age.

Methods The study was carried out in a sample of 291 students (113 boys and 178 girls). The NPC, far and near horizontal phorias (FHP and NHP) and far and near positive and negative fusional vergences, break (FPFV-b, FNFV-b, NPFV-b, NNFV-b) and recovery (FPFV-r, FNFV-r, NPFV-r, NNFV-r) were obtained using a visual acuity card for near distance and a ruler (in mm) for the measurements of the NPC, and a near visual acuity card, a Snellen chart for far distance and a prism bar for the measurements of the phorias and the fusional vergences.

Results The mean age of the sample was 12.40 years, in a range from 16.42 years to 9.42 years. The interaction between NPC and age showed that this value tended to recede with the age ($b=0.054$, $t=3.86$, $p<0.001$). Regression slopes were performed to investigate the correlation between mean values of each condition (NPC, FHP, NHP, FPFV-b, FNFV-b, NPFV-b, NNFV-b, FPFV-r, FNFV-r, NPFV-r and NNFV-r) and age. The analysis of the results of the horizontal phorias showed that exophoria decreased and esophoria increased with age both at far ($b=0.025$, $t=2.46$, $p=0.014$) and near distance ($b=0.976$, $t=4.32$, $p<0.001$). The analysis of the fusional reserves showed that none of them varied significantly with age ($p>0.05$ in all cases).

Conclusion The NPC and horizontal phorias vary with the age. Specifically, NPC recedes with age, exophoria tends to diminish, whereas esophoria tends to increase with age. In the other hand, fusional vergences do not vary significantly with age.

■ 404

Atypical blepharospasm

VILLAFRUELA I (1), SAAVEDRA J (2)
(1) *Ophthalmology, Madrid*
(2) *Medicine, Madrid*

Purpose Benign essential blepharospasm is a focal cranial dystonia characterized by bilateral involuntary spasms of the muscles of the eyelids (pretarsal, preseptal, and periorbital orbicularis oculi) and the upper face (corrugator, procerus), in the absence of other ocular or adnexal disease.

Methods We present a woman of 61 years old, who complained of intense photophobia, ocular discomfort and dry-eye symptoms. These symptoms were bilateral; she reported that she had given up watching television and reading. She was anxious and depressed. There was no family history of dystonia or blepharospasm. We didn't find any other dystonic movement of facial muscles. Pharmacologic (cholinesterase inhibitors), ice pack test, electrophysiologic and immunologic tests (antiacetylcholine receptor, anti-MuSK) to rule other neurological diseases were normal.

Results She was managed with sunglasses, dry eye and blepharitis topic treatment and botulinum toxin injections in the orbicular muscle every 3 to 5 months during the first five years. After two years the treatment was not so effective. At the present, combined treatment with Botulinum toxin, dopaminergic and antipsychotic drugs allows an acceptable functional status allowing her to manage with daily activities with a discrete familiar supervision.

Conclusion The diagnosis of BEB is based on exclusion. Treatment with botulinum toxin type A injections prevent or minimizes eyelid spasms in more than 90% of patients, but only on a temporary basis. The bizarre nature of the spasms, their fluctuating course, and the confounding associations between patient's emotional status and the spasms often lead to wrong diagnosis of psychopathologic etiology of blepharospasm. The therapeutic approaches are symptomatic drugs and botulinum toxin injections.

■ 405

Particularities of pupillograms in children and adults with hypermetropic amblyopia

BUSHUYEVA NN, BOYCHUK IM, SHAKER M.H.DUHAIR MH
Filatov Institute of Eye Diseases and Tissue Therapy, lab.of binocular disorders, Odessa

Purpose It is known that disorders of afferent pupil light pathways in patients with amblyopia are rather frequent that's why the goal of the work was to study pupillogram particularities in amblyopic patients and compare them with healthy persons.

Methods : The pupillographic method was performed in 17 healthy persons aged 17–19 and in 45 patients aged 3-14 with hypermetropic amblyopia. Methods of research included direct, consensual and accommodation-convergence reaction of pupils to flash; the dynamics of pupil size change in time during accommodation; visual acuity testing for near and far distances, refractometry.

Results There were direct correlation between visual acuity and pupil amplitude, speed, fluctuation frequencies of the pupil area during all types of pupil reactions (for direct flash, consensual and accommodative-convergent reaction) expressed differently in periods. It was found that latent period restoring of pupil's size after direct flash reaction was longer on the both eyes in patients with hypermetropic amblyopia in comparison with healthy persons. During consensual reaction these patients had significant difference between pupil's area of both eyes (70%), though healthy persons have equal pupil's area after direct flash. During accommodative pupil reaction pupil maximal square is less and latent period of recovery is larger in amblyopic patients independently from fixation than in healthy persons

Conclusion The analysis of pupillograms may be used as objective test in the diagnosis of lesions along the pupil and visual pathway in patients with hypermetropic amblyopia

■ 406

Results of optical coherent tomography of retina and optical nerve in children with unilateral amblyopia of high degree

BOYCHUK IM (1), IVANITSKAYA EV (2)
(1) *Filatov Institute of Eye Diseases and Tissue Therapy, Odessa*
(2) *Filatov Institute of Eye Diseases and Tissue Therapy, dep. of retinal and optic nerve diseases, Odessa*

Purpose There are controversial results in OCT studies of amblyopic patients. To find any differences between fellow and amblyopic eyes in amblyopic patients of different kind the investigation of structural peculiarities of retina and optic nerve in children with unilateral amblyopia of high degree was conducted

Methods 18 anisometropic amblyopes and 19 unilateral strabismic amblyopes with equal high hypermetropia aged 6-12 y.o. were studied with the help of Stratus OCT Model 3000 Carl Zeiss Meditec, Inc. Thickness of foveal retina, central part of retina in 9 segments, thickness of peripapillary retina and RNFL (retinal nerve fiber layer), parameters of optic nerve were evaluated on amblyopic and fellow eyes in both groups.

Results Average foveal thickness in both amblyopic and fellow eyes was equal in both groups. Some differences were found between amblyopic and fellow eyes in patients with anisometropia (thickness of external superior segment of retina and average thickness of peripapillary RNFL of amblyopic eyes were larger than in fellow eyes). Differences between groups were found in fellow eyes of children with strabismic and anisometropic amblyopia by structural indices of optic nerve.

Conclusion Preliminary results show necessity of further studies on the greater clinical material.

■ 407

Visual evoked potentials (VEPs) in early diagnosis of myelomeningocele in infants

SOBIESZCZANSKA M (1), PILECKI W (1), JANOWA A (2), SALOMON E (2),
GUTH C (1)

(1) *Department of Pathophysiology, Wroclaw Medical University, Wroclaw*
(2) *Department of Physiology, Wroclaw Medical University, Wroclaw*

Purpose Myelomeningocele (MMC) is a serious congenital anomaly of the central nervous system, complicated sometimes by visual impairment. It requires rapid diagnosis, for which VEPs can be useful. Aim of the study was to assess MMC influence on visual pathway functioning using flash VEPs.

Methods VEPs with 1 Hz flash stimulus were recorded in 36 infants aged 1-52 weeks with diagnosed MMC within spinal cord, accompanied by hydrocephalus, without abnormalities in routine ophthalmologic examination. Analysis of the VEPs concerned waveforms morphology and P100 peak latency.

Results The quite normal VEPs were observed in 15 of 36 (41.7%) examined children. In the left 21 children, the VEPs tracings were found to be illegible, with no discernable P100 peak. Among the 15 VEPs results of normal morphology, the P100 latency fell into the lab limits, i.e. 105–145 ms, in 13 of 36 cases (36.1%), whereas in the left 2 cases (5.6%), the slightly delayed P100 latency was noted, i.e. 148 ms and 164 ms. In 15 of 36 examined children, with the typical VEPs waveforms, the P100 latencies were referred to the age-matched control group. The mean P100 latency was 130.3 ± 14.7 ms and 124 ± 9.8 ms, respectively. The inter-group difference appeared to be statistically insignificant. In general, the abnormal VEPs results were observed in 63.9% of the all patients with MMC – 58.3% had the illegible VEPs and 5.6% had the delayed P100 latency.

Conclusion VEPs can be a complementary method in children with MMC, useful in detecting impairment of visual system functioning.

■ 408

Time dynamic of visual field progression in patients with drusen of the optic disc compared to patients with normal tension glaucoma and frequency of visual field progression in patients with drusen of the optic disc

SCHARGUS M, GRAMER G, GRAMER E
University Eye Hospital, Wuerzburg

Purpose To investigate the time dynamic of visual field progression (VFP) in patients with optic disc drusen (ODD) compared to patients with normal tension glaucoma (NTG) and to evaluate the age dependence of the stage of visual field loss (VFL) and the frequency of VFP in ODD.

Methods VFL at time of diagnosis was staged in 144 ODD and 115 NTG patients. According to the eye with the more severe VFL we categorised for both diseases 3 groups: no VFL, VFL stage I/II, stage III/IV(classification of Auhorn). Visibility of ODD at time of diagnosis was staged. 46 patients (80 eyes), categorized in 4 age groups, had a perimetric follow up of 21 and more months. Spearman-Rho Test was used for statistics.

Results I. ODD: The time difference between the mean age at diagnosis of 28 patients with no VFL and 32 patients with VFL stage I/II was 6.8 years, NTG: The time difference between the mean age at diagnosis of 35 patients with preperimetric NTG and 45 patients with stage I/II was 8.3 years.II. With increasing age at time of diagnosis there was an increasing visibility of ODD ($p<0.01$) and an increasing frequency of more advanced stages of VFL ($p<0.01$).III. 31 of 80 eyes (39%) showed a VFP during a mean observation time of 84 months. There was within the 4 age groups an age related increase of VFP (17% vs 29% vs 39% vs 59%) ($p<0.01$)

Conclusion Patients with ODD have a faster initial VFP than patients with NTG. The age dependent VFP is confirmed in our prospective pilot study: the older the ODD patient the higher the stage of VFL and the more frequent the VFP. Age is a risk factor in both diseases.

■ 409

Early diagnosis of compressive optic neuropathy (NOC) in patients with Thyroid Autoimmune Ophthalmopathy (TAO)

CARNOVALE SCALZO G (1, 2), LABONIA AF (2), PAOLA A (1, 2),
 SCORCIA V (1, 2), SCORCIA GB (1, 2), BRUZZICHESSI D (1, 2), COSTANTE G (2),
 SCORCIA G (1, 2)

(1) Eye Clinic, University of Catanzaro "Magna Græcia", Catanzaro
 (2) Department of Sperimental and Clinic Medicine, University "Magna Græcia",
 Catanzaro

Purpose Thyroid Autoimmune Ophthalmopathy (TAO) is a disfiguring and disabling autoimmune disease. Severe ophthalmopathy (3-5%) may result in compressive optic neuropathy (NOC), with possible sight loss. Aim of this study: the evaluation of early morphological and functional alterations of the optic nerve in TAO patients without clinically evident signs of NOC (i.e. visual acuity defect), by recently available techniques and to correlate the results to the Clinical Activity Score.

Methods We examined subclinical optic nerve involvement of 82 eyes in 41 consecutive TAO patients (31 women and 10 men, age 30 to 70 years). All patients have been submitted to: clinical and instrumental evaluation (perimeter computerized, OCT and GDx). Criteria of exclusion: ocular pathologies associated to impairment of visual acuity (pathologic myopia, cataract, diabetes, not autoimmune optic neuropathy).

Results 19% of the patients did not present perimetal defects, 81% showed a variety of perimetal defects (reduction of the foveal sensitivity, para- pericentral scotomas). Alterations at OCT examination occurred in 65% of the cases, while 50% of the patients presented alterations of the nervous fiber thickness analyzed by GDx. These alterations were correlated to the degree of TAO activity.

Conclusion Our study indicates that 1) in TAO patients, functional optic nerve alterations occur at an early stage, before any anatomical damage; 2) OCT is more sensitive than GDx analysis for NOC detection.

■ 410

Severe maculopathy and leukoencephalopathy in a young patient

WILLERMAIN F (1), DIELMAN C (2), BRADSTREET C (2), HOUA N (3),
 KAMPOLIRIDIS S (4), CASPERS L (1)

(1) Ophthalmology, CHU St-Pierre, Bruxelles
 (2) Medicine, CHU St-Pierre, Bruxelles
 (3) Neurology, CHU St-Pierre, Bruxelles
 (4) Radiology, CHU St-Pierre, Bruxelles

Purpose To describe the unusual presentation of a severe maculopathy in the setting of progressive leukoencephalopathy

Methods A 24 year old man from Pakistan presented to our consultation for sudden loss of vision in his left eye. He reported that he had acutely lost the vision of his right eye 2 weeks earlier. The past medical history was significant for a transient hemiplegia two years before. Visual acuity was limited to visual perception in both eyes.

Results Slit lamp biomicroscopy was normal. Fundoscopic examination revealed an atrophic macula with a salt and pepper appearance in the right eye and a yellowish coloration of the macular area of the left eye. Fluorescein angiogram showed an important leakage of the dye at the posterior pole in the left eye. Optical coherence tomography demonstrated an atrophic macula in the right eye and a neuroretinal detachment with retinal cysts in the left eye. ERG was severely decreased in both eyes. High doses steroids were given but vision continued to deteriorate. An extensive work-up was negative with a normal CNS MRI and the patient was discharged. One month latter he presented with a left hemiplegia. Few days after, his mental status begun to deteriorate. CNS MRI showed a very severe leukoencephalopathy. Genetic testing and muscle biopsy was not in favor of MELAS. Skin biopsy showed the presence of abnormal material within the basement membrane of vascular smooth cells.

Conclusion Although our patient had characteristics compatible with different described diseases (MELAS, CADASIL ...), several features render the diagnosis uncertain. It is thus possible that he presented a rare form of an unknown sporadic retino-cerebral angiopathy.

■ 411

Microhaemodynamics in patients with primary open angle glaucoma

SHAMSHINOVA AM (1), BAKHINSKI P (2)
 (1) Moscow Helmholtz Institute of Eye Diseases, Laboratory of Clinical Electrophysiology
 for Vision, Moscow
 (2) Clinical Hospital of District of Vidnoe, Eye Department, Vidnoe

Purpose To investigate the eye and general microhemodynamics in primary open angle glaucoma (POAG) with normalized intraocular pressure.

Methods The eye and general microhemodynamics were investigated in 29 patients with primary open angle glaucoma (POAG) with normalized intraocular pressure. A computer laser Doppler fluorimetry LAKK-01 (NPO "Lasma", Moscow) was used. The occlusion and respiratory tests were applied to estimate the adrenergic regulation of bringing branch of the common micro- blood flow

Results In patients with POAG two types of eye micro- blood flow changes were determined. One of them was hyperemic type with increase of a micro- blood flow parameter, another one was the spastic type with reduced micro- blood flow parameter. The statistically reliable differences of the amplitudes of the active and passive regulation mechanisms of the common micro blood- flow were marked in the revealed groups. The decrease of the common adrenergic activity of bringing arterioles was determined in patients with hyperemic type of eye microcirculation disturbance. The cause of these was the reduction of neurogenic tone with simultaneous spasm formation during functional test. The relative spasm disposition or the bringing micro vessels spasm was recorded in patients with POAG and spastic type of eye microcirculation disturbances. The micro vessels spasm was amplified while carrying out the functional test.

Conclusion We suppose that the revealed data indicate different vascular pathogenetic mechanism development and current glaucomatous process in the specified groups of patients.

■ 412 / 2456

Community optometrist referrals for glaucoma- referral accuracy and outcomes

MALIK ANJ, YUEN L, ALTHAUSER S
 Royal Free Hospitals NHS Trust, London

Purpose The UK government commissioned National Eye Care Services report recommends changes to allow direct referrals by optometrists to hospital services, as well as new roles in community care of glaucoma patients. We aimed to determine optometrist referral patterns and the outcomes.

Methods Retrospective study of new optometrist referrals to a specialist glaucoma clinic over a six month period

Results The positive predictive value (PPV) of a positive outcome (diagnosis with glaucoma or as suspected glaucoma) was 0.35, based on their first visit (total number patients 102). Referral based on one suspicious finding occurred in 67% of patients (n=68). The most common reason stated for referral was raised intraocular pressure (IOP) in 60% of cases. The mean IOP for referral was 24mm Hg (range 13-35). In comparison the mean IOP of this subgroup was 17mm Hg (range 12-28) when measured in hospital ($p<0.001$). Non contact tonometry was used by optometrists in 96% of patients. Other causes for referral included suspect looking optic disc (44%) and visual field loss (22%). Visual field defects were found in 40% (23/57) of patients by optometrists, when performed. In patients referred with visual field defects 39% (9/23) had the defect confirmed at the hospital visit. The mean cup disc ratio was comparable between optometrists and the hospital glaucoma service ($p=0.09$). 24% of referrals documented a family history.

Conclusion The PPV of referral accuracy improves when more than one suspicious finding is documented. Non- contact tonometry and perimetry results from a single patient visit is a major source of unnecessary referrals. Emphasis on specific training and equipment of community optometrists will be required as their role in management of glaucoma increases.

■ 413 / 2457

Development of an Instrument to Assess Behavior and Readiness for Behavior Change in Patients Taking Ocular Hypotensive Therapy

SCHWARTZ GF (1), PLAKE K (2), MYCHASKI W MA (3)

(1) Glaucoma Consultants, Wilmer Eye Institute, Johns Hopkins University, Baltimore
(2) Purdue University School of Pharmacy and Pharmaceutical Sciences, West Lafayette
(3) Pfizer Inc, New York

Purpose To develop and conduct preliminary validation of a tool to assess medication-taking behavior in patients taking ocular hypotensive therapy and readiness for behavior change using the Transtheoretical Model.

Methods A survey was developed with questions derived from a review of the ophthalmic and non-ophthalmic literature and modified to apply to glaucoma or ocular hypertensive patients. The questionnaire included patient evaluations of health and medications, difficulties in taking ophthalmic medications, use of medications, visual function (NEI-VFQ 25), and demographics. The draft was reviewed for content and face validity by a panel of 8 glaucoma specialists and behavioral and health economics experts.

Results The panel confirmed the content and face validity of the questionnaire. The panel recommended reducing forced choices by adding more coded responses for selected items and amending wording to improve readability and response clarity. Additional testing in the target population is planned prior to use in patient care settings.

Conclusion The expert panel provided preliminary validation of the instrument. Application in patient care settings may allow physicians to identify medication-taking behavior and patients in whom adherence or persistence needs to be improved. Identifying patients' behaviors and issues in taking medications will allow appropriate interventions to be designed.

■ 414

Effect of Nerve Growth Factor on Retinal Ganglion Cell Loss in Ocular Hypertension Model of Rats

YEOM HY (1), KIM SG (2), MA KT (2), KIM CY (2), SEONG GJ (2)

(1) Siloam Eye Hospital, Seoul

(2) Dept. of Ophthalmology, Yonsei University College of Medicine, Seoul

Purpose To investigate whether intravitreally injected nerve growth factor (NGF) can reduce the loss of retinal ganglion cells in ocular hypertension model of rats.

Methods Sixteen Sprague-Dawley rats with cauterization of 3 episcleral vessels on their right eye for chronic glaucoma model, were divided into one vehicle group and three NGF groups, each receiving intravitreal injection of 0.1, 0.5, 1 µg/ml NGF. During 6 weeks, Intraocular pressure of both eyes was measured at baseline and every week, and NGF and vehicle were injected at 4 weeks. Before injection and at 6 weeks, retinal function was monitored by electroretinography (ERG). After 6 weeks, retinal ganglion cells were counted by labeling with dextran tetramethylrhodamine (DTMR) in three rats and glial fibrillary acid protein (GFAP) staining was performed in a rat in each group.

Results Comparing with untreated fellow eyes, intraocular pressure was elevated 27~73% at 6 weeks and retinal ganglion cell counts were decreased 23%, 22%, 13%, 20% in vehicle group and 0.1, 0.5, 1 µg/ml NGF groups respectively. 0.5 µg/ml NGF group showed statistically significant survival effect. GFAP was most strongly stained in vehicle group and least in 0.5 µg/ml NGF group. ERG amplitude (b-wave/a-wave) improvement was reported only one eye of 0.5 µg/ml NGF group among all experimental eyes.

Conclusion Intravitreally injected NGF may present neuroprotection on retinal ganglion cells in chronic hypertensive rat eyes.

■ 415

Cellular Response of Astrocytes to Increased Intraocular Pressure in Rat Glaucoma Model

NIITYKOSKI M (1), KALESNYKAS G (1), RANTALA J (1), MIETTINEN R (2), SALMINEN A (2), KAARNIRANTA K (1), UUSITALO H (1, 3)

(1) Department of Ophthalmology, University of Kuopio, Kuopio

(2) Department of Neuroscience and Neurology, University of Kuopio, Kuopio

(3) Department of Ophthalmology, Kuopio University Hospital, Kuopio

Purpose Human and experimental studies have found morphological changes and alterations in the expression of glial fibrillary acidic protein (GFAP) in astrocytes of glaucomatous retinas. One typical factor induced by stress is synthesis of heat shock proteins (HSPs) that helps to maintain survival and functionality of cells. Therefore, the aim of the present study was to examine quantitative changes of retinal astroglial cell population using stereology and response to stress induced by elevated intraocular pressure (IOP).

Methods The IOP of one eye of adult male Wistar rats was elevated by laser photoocoagulation of episcleral and limbal veins. A week later, one group of rats was sacrificed and the laser treatment was repeated in the remaining of rats that were sacrificed 2 or 3 weeks after the first laser treatment. Thereafter, retinal astrocytes and HSP25 positive cells were visualized from flat-mounted retinas immunohistochemically. The GFAP and HSP25 colocalization was confirmed using laser scanning confocal devide. The total number of retinal astrocytes was estimated using stereology.

Results The laser treatment induced an increase in the IOP as compared to non-laser-treated control eyes (the first week: 31.3±11.5 mmHg, the second week: 29.4±15.0 mmHg, the third week: 24.0±6.7 mmHg, mean±SD). Stereological counting indicated that laser-treated eyes had a decreased number of GFAP-immunoreactive cells by 42% after 3 weeks of elevated IOP. Furthermore, there was a 3-fold increase in the number of astrocytes that colocalize HSP25 in laser-treated eyes than that in controls.

Conclusion The present study demonstrates that the retinal astrocytes are damaged in response to elevated IOP.

■ 416

Comparison of simultaneous readings of intraocular pressure in rabbits using Perkins' hand-held, Tono-Pen XL and TonoVet tonometers

KALESNYKAS G (1), KÄSNÄNEN H (1, 2), TERÄSVIRTA M (1, 2), UUSITALO H (1, 2)

(1) Department of Ophthalmology, University of Kuopio, Kuopio

(2) Department of Ophthalmology, Kuopio University Hospital, Kuopio

Purpose To compare intraocular pressure (IOP) measurements of Perkins' hand-held (Clement Clarke Ltd, UK), Tono-Pen XL (Mentor, USA) and TonoVet (Tiolat Oy, Finland) tonometers using *in vivo* manometry.

Methods The IOP was set and measured manometrically in anesthetized (a mixture of ketamine, 25 mg/kg and medetomidine, 0.3 mg/kg, im) adult New Zealand White rabbits (n = 2, weight ~3.5 kg) after eye anterior chamber cannulation through the peripheral cornea with a 26-gauge needle connected to a vertically adjustable reservoir. The IOP was raised and lowered in approximately 5 mmHg steps from 5 mmHg to 50 mmHg using open stopcock mode. The IOP was measured with Perkins' hand-held, Tono-Pen XL and TonoVet tonometers by taking 4-6 readings at each point.

Results Perkins' hand-held tonometer showed the highest and Tono-Pen XL the lowest accuracy in the IOP measurements. Moreover, Tono-Pen XL had the highest variation in the IOP measurements.

Conclusion The results of our experiment suggest that Tono-Pen XL which is widely used in animal research is subjected to higher bias towards the true IOP measurements than Perkins' hand-held tonometer or TonoVet.

■ 417 / 2256**Inducible NOS synthase in the human trabecular meshwork (TM) from patients with primary open-angle glaucoma (POAG)**

FERNANDEZ DURANGO R (1), GARCIA FEIJOO J (2), CASTILLO A (2), MARTINEZ DE LA CASA JM (2), GARCIA-BUENO B (3), FERNANDEZ-CRUZ A (4), LEZA JC (3)

(1) Unidad de Investigación, Hospital Clínico San Carlos, Madrid
 (2) Ophthalmology, Hospital Clínico San Carlos, Madrid
 (3) Pharmacology, Complutense University, Madrid
 (4) Internal Medicine, Hospital Clínico San Carlos, Madrid

Purpose Visual field damage and IOP in patients with POAG has been correlated with inflammatory parameters in TM. Constitutive and inducible nitric oxide synthase (NOS) activities and expression were analyzed in TM.

Methods TM specimens were obtained at filtration surgery from 60 glaucoma patients. Constitutive and inducible (iNOS) activities were measured by the conversion of L-[14C]-arginine to L-[14C]-citrulline method. NOS expression was detected by real-time RT-PCR. Levels of malondialdehyde (MDA), were evaluated in the aqueous humor (AH) of control (cataract n=16) and POAG patients (n=17). Patients underwent a Octopus TG1 visual field examination and tonometry before surgery.

Results Constitutive, Ca²⁺-dependent activity of the TM from the patients with MD between 2 and 12 dB was similar (120 ± 60 pmol·min⁻¹ mg prot⁻¹) but not detectable in the patients with MD>12 dB. No differences were found on the expression of the main constitutive isoform in TM (eNOS). iNOS mRNA and activity were only detected in the TM of patients with MD>12 dB (396 ± 56 pmol·min⁻¹ mg prot⁻¹). MDA levels correlated with iNOS activity (control: 0.05 ± 0.003 nmol/ml; POAG: 0.07 ± 0.001 nmol/ml; p<0.05). No other correlations were found.

Conclusion The expression and activity of the high-output source of NO are increased in the TM of POAG patients with severe visual field damage, an effect that is paralleled with MDA accumulation in AH. Constitutive NOS activity is reduced as the severity of POAG increases. These results suggest that excessive synthesis of NO produced by iNOS may damage TM cells and increases the severity of the loss of the visual field in these patients.

■ 419**Efficacy and safety of selective laser trabeculoplasty (SLT) in a busy clinic setting**

BYARDS S (1), ANSARIE A (1, 2)

(1) Eye Department, Eye Ear and Mouth Unit, Maidstone

(2) Department of Physical Sciences, University of Kent, Canterbury

Purpose To assess the short term efficacy and safety of selective laser trabeculoplasty (SLT) in the treatment of primary open angle glaucoma (POAG) in a busy glaucoma clinic setting.

Methods Patients were recruited from general and glaucoma clinics at Maidstone Hospital and Pembury Hospital. The indications for treatment were:

- POAG requiring further IOP reduction to target level
- POAG patients developing drop intolerance
- POAG patients opting against filtering surgery

Patients with primary angle closure and inflammatory glaucoma were excluded. All patients received SLT treatment with the Laserex Tango machine (Haag-Streit, UK), treatment being applied over 360 degrees with a mean 108 applications (range 90-120), power 0.5-1.1mJ depending on the degree of trabecular pigmentation. Follow-up was arranged at 1 week, 1 month and 3 months post-treatment. Success was regarded as reaching the target IOP with or without reduction in topical medications.

Results A total of 28 patients (37 eyes) were treated. SLT produced a statistically significant reduction of IOP at each follow up point (p<0.05). The mean IOP reduction at 3 months was 3.6mmHg (18% reduction) compared to baseline. All patients reached the desired target IOP; however the original topical medications were continued. 8 patients (29%) reported some mild discomfort, but this was temporary, lasting less than 24 hours.

Conclusion SLT was a safe and efficacious adjunctive treatment for POAG, and was easy to administer in a typical, busy clinic situation. All patients reached the desired target IOP; however, topical medications were continued to maintain this level of IOP.

■ 418**Selective laser trabeculoplasty in pseudoexfoliation secondary glaucoma**

CELLINI M, LEONETTI P, ZAMPARINI E, CAMPOS EC
Department of Surgery and Transplant, Bologna

Purpose The pseudoexfoliation glaucoma (PEXG) is characterized by the accumulation of abnormal fibrillar materials and pigmented cells in trabecular meshwork. We evaluated if SLT is an effective method to decrease the intraocular pressure (IOP) in patient with PEXG and if it can change the MMP-3/TIMP-1 ratio.

Methods We enrolled 20 patients with PEXG, for a total of 40 eyes. The patients had a bad intraocular tensional compensation under maximal medical therapy. The eyes with worst visual field were treated with trabeculectomy while the other eyes with SLT. The aqueous humor was aspirated during surgery and the concentration of MMP-3 and its inhibitor TIMP-1 was assessed. SLT was performed using 50 spots with a size of 400 µm, pulse duration of 3 ns with mean power of 0.572 ± 0.115 mJ. One month after SLT, because the reduction of IOP wasn't low enough (at least 20% lower than the starting IOP) the patients were treated with trabeculectomy and the MMP-3/TIMP-1 ratio was evaluated.

Results At the baseline and after 15 days from SLT, the IOP resulted to be 25.8 ± 1.9 vs. 23.0 ± 1.9 mmHg (p<0.001) but 30 days after the treatment, the IOP rose to 25.4 ± 1.6 mmHg. (p<0.591). The MMP-3 rate in the eyes treated with SLT was 1.817 ± 0.135 ng/mL while the rate quoted in the other control eyes was 1.909 ± 0.205 ng/mL. (p<0.177). The TIMP-1 rate was 962.3 ± 33.971 ng/mL and 972.3 ± 24.662 ng/mL. (p<0.264) respectively. Before and after SLT treatment MMP3/TIMP-1 ratio resulted to be 0.19.

Conclusion Our findings show that the MMP-3/TIMP-1 ratio is not modified by SLT and for this reason the SLT is not effective to treat pseudoexfoliation glaucoma. Nevertheless our results need to be verified in a greatest number of PEXG patients.

■ 420**Topical plus subconjunctival versus retrobulbar anesthesia in non perforant sclerectomy supplemented with 5-FU**

GUTIERREZ C, TEUS MA, BOLIVAR G
Glaucoma, Alcalá de Henares, Madrid

Purpose To compare the safety and efficacy of topical plus subconjunctival versus retrobulbar anesthesia for primary non perforant sclerectomy supplemented with 5-Fu.

Methods A prospective randomised study of 30 patients who were randomized to receive subconjunctival (n = 14) or retrobulbar (n = 16) anesthesia. Operating conditions, patient comfort, postoperative pain, total pain and surgical outcome were evaluated.

Results There were no differences in operating conditions. The retrobulbar group reported significantly more pain during administration of the anesthetic agent than the topical group (P= 0.00). The subconjunctival group reported more discomfort during surgery than the retrobulbar group (P= 0.00), however this feature was not a problem for the surgeon. No significative differences were found in the postoperative pain in both groups. No significative differences were found between groups in the total pain. No significative differences were found in the success rate between both groups.

Conclusion Topical anesthesia is a safe and effective alternative to retrobulbar anesthesia for non perforant sclerectomy supplemented with 5-FU.

■ 421 / 4457

The safety and efficacy of trabeculectomy with mitomycin C (MMC) in juvenile open angle glaucoma

CHIANG MY, LOCKWOOD A, WILLIAMS TA, JAYAPRAKASAM A, SII F, SHAH P
Birmingham and Midland Eye Centre, Birmingham

Purpose To determine whether trabeculectomy with MMC can be used safely and effectively to control the intraocular pressure (IOP) in patients with severe refractory juvenile open angle glaucoma (JOAG).

Methods This study emerged as part of Phase 3 of the Birmingham ReGAE (Research into Glaucoma And Ethnicity) Project- a prospective and consecutive interventional case series investigating the surgical outcomes of trabeculectomies with MMC. There are currently 226 eyes in this actively updated database. Patients with JOAG were selected for the study.

Results Twenty-three eyes of 15 patients with JOAG were included. Ten out of 23 eyes (43.5%) were African-Caribbean eyes and 5 eyes (21.7%) had previous trabeculectomy. The mean age at surgery was 30.96 (range 11 – 48). The mean duration of follow-up was 633 days (range 90 – 2172 days). Twenty-two eyes (95.7%) achieved IOP of ≤ 21 mmHg at the latest follow-up and 19 eyes (82.6%) did not require any medication. Fourteen eyes reached 1 year follow-up. Success at 1 year stratified by IOP were 92.9% (n=13) at IOP ≤ 21 mmHg and ≤ 17 mmHg and 85.7% (n=12) at IOP 14 mmHg. An IOP reduction of ≥ 30 % were obtained in 92.9% (n=13). The mean IOP decreased significantly from 23.36±2.69 mmHg pre-operatively to 13.21±1.51 mmHg at 1 year ($p<0.05$). No visual loss of > 2 Snellen lines occurred. Three eyes (13%) had clinically significant early hypotony needing intervention. One eye (4.3%) developed a late bleb leak requiring bleb revision. One eye (4.3%) required tube surgery at 1 year.

Conclusion Trabeculectomy with MMC can be used safely and effectively to control IOP and preserve vision in refractory juvenile open angle glaucoma.

■ 422

Trabeculectomy with Mitomycin C – Safety and Efficacy for patients with Normal Tension Glaucoma

LOCKWOOD A, CHIANG MY, SII F, WILLIAMS TA, SHAH P
Birmingham and Midland Eye Centre, Birmingham

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 normal tension glaucoma (NTG).

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 MMC. Twenty seven eyes of 20 patients with NTG were included. Incomplete IOP control was deemed when IOP reduction was less than 25 % of the preoperative mean on two successive occasions.

Results The mean length of follow up from the time of surgery to the last visit was 23.4 months (range 1 – 60 months). At the last follow up examination 22 (78 %) of the trabeculectomies achieved complete IOP control. Eighteen trabeculectomies reached 12 months follow up and of these 17 (94 %) achieved an IOP of 14 mm Hg or less and 11 (61 %) achieved an IOP reduction of 30 %. One (3.7 %) trabeculectomy failed and required a repeat trabeculectomy. No patients required IOP lowering medication postoperatively. Best-corrected visual acuity improved or remained within two lines of preoperative visual acuity in 26 eyes (96 %). No patients developed late bleb leaks or late hypotony.

Conclusion Trabeculectomy with intraoperative application of MMC can be used with good efficacy and with few complications in patients with NTG

■ 423 / 4458

Excimer laser trabeculotomy (ELT) - an alternative laser treatment to reduce intraocular pressure

WILMSMEYER S (1), PACHE M (1), FUNK J (2)

(1) University Eye Hospital, Freiburg

(2) Charlottenklinik, Stuttgart

Purpose Excimer laser trabeculotomy (ELT) is a minimally invasive surgical technique to reduce intraocular pressure (IOP) in patients with glaucoma or ocular hypertension. Our purpose was to examine IOP-reduction in patients treated with ELT alone or ELT combined with cataract surgery.

Methods To increase the outflow of aqueous humor, 10 microperforations of the trabecular meshwork were performed by an endoscope guided photoablative laser probe (Excimer laser, AIDA, TUI-Laser, Munich). In our retrospective study one group of patients received an ELT alone, the other group underwent combined surgery consisting of phacoemulsification + ELT. IOP, visual acuity and antiglaucoma drugs (AGD) were determined preoperatively (T0) and 2-4 (T1), 5-7 (T2), 10-14 (T3) and 21-27 (T4) months after surgery.

Results IOP in eyes with ELT alone was reduced from 24.1 (n=69) mmHg preoperatively to 18.8 (T1, n=67), 19.9 (T2, n=60), 18.9 (T3, n=49), 19.4 (T4, n=43) mmHg resp.. The number of AGD was 1.9 (T0), 1.2 (T1), 1.4 (T2), 1.9 (T3), 1.4 (T4). In the phacoemulsification + ELT group, an IOP-reduction from 22.4 mmHg (T0, n=58) to 16.5 (T1, n=55), 16.2 (T2, n=49), 16.2 (T3, n=51), 15.6 (T4, n=38) mmHg resp., was observed. The number of AGD showed no significant change (1.2 (T0), 0.9 (T1), 1.1 (T2), 1.2 (T3), 1.1 (T4)).

Conclusion ELT, especially in combination with phacoemulsification, is a promising laser treatment to reduce IOP for at least 1-2 years.

■ 424

The Macula in Glaucoma

CILLIERS H (1,2), FRANZCO GB (3), FRANZCO DM (4), FRANZCO MC (5,2), LAMOLIREUX E (2)

(1) Dept of Ophthalmology, Northern Hospital, Melbourne

(2) Centre for Eye Research Australia (CERA), Melbourne

(3) Dept of Ophthalmology, Cheltenham General Hospital, NHS Foundation Trust, Gloucestershire

(4) Rotorua Eye Clinic, Rotorua

(5) Melbourne Eye Specialists, St Vincent's Private Hospital, Melbourne

Purpose Retinal nerve fibre layer (RNFL) loss precedes visual field (VF) loss in glaucoma. Zeimer confirmed that there is significant correlation between decreased macular thickness and glaucoma. To measure and describe macular changes in 356 eyes with glaucoma and ocular hypertension.

Methods Retrospective case analysis of patients all whom had ophthalmic examination, BCVA, keratometry, optic disc photography, standard automated perimetry (SAP) with an Octopus Perimeter and macular thickness mapping by the Retinal Thickness Analyser (Talia). Patients were grouped into Ocular Hypertension (OHT), Pre-perimetric Glaucoma (PPG), Early Glaucoma (EG), Moderate Glaucoma (MG) and Advanced Glaucoma (AG). The mean was calculated between each group using Bonferroni/Dunn post hoc test and correlation between VF and RTA by linear regression analyses.

Results Macular changes on RTA may be described as follows: focal erosion, hemi-field flattening, steepening of the outer rim, foveal widening, and overall flattening. Groups were well aged matched, except groups 1-OHT and 2-PPG, mean age 60 and 62.7 years respectively, with statistically significant age difference ($p=0.027$). The trend for mean deviation (MD) and loss variance (LV) to match glaucoma progression was statistically significant except groups 1-OHT and 3-EG with regards to MD and groups 1-OHT, 2-PPG and 3-EG re LV. The trend for decreased Posterior Pole (PP) and Peri-foveal (PF) thickness (μm) as glaucoma progressed was not statistically significant, however correlation between VF and RTA values was statistically significant.

Conclusion RTA reliably maps structural changes in the macula in glaucoma which have a functional correlate with VF. Glaucoma described in terms of macular change necessitates further longitudinal studies.

■ 425

Correlation between perimetric indices of conventional automated perimetry and structural papillary parameters obtained by means of OCT

LOPEZ PENA MJ, FERRERAS AMEZ A, LARROSA JM, POLO V, BORQUE E, HONRUBIA FM
Ophthalmology Department, Zaragoza

Purpose To evaluate the relationship between standard automated perimetry (SAP) and the structural parameters of the optic nerve head performed by means of optical coherence tomography (OCT).

Methods A total of 423 subjects were included in the study. Eyes were classified into four diagnostic groups based on intraocular pressure value, optic nerve head appearance (stereo-photographs) and SAP results: 87 normal subjects, 192 ocular hypertensive eyes, 70 pre-perimetric glaucomas and 74 glaucomatous patients. All of them underwent a full ophthalmic exploration, at least 2 SAPs (Humphrey 24-2 SITA standard) and an OCT evaluation (Stratus OCT 3000). Pearson's correlations were calculated between global perimetric indices of SAP (mean deviation –MD- and corrected pattern standard deviation –CPSD-) and optic nerve head parameters.

Results Mild to moderate significant ($p<0.05$) correlations were found between several optic nerve head parameters and perimetric indices, in the glaucoma group. However, the rest of the groups showed mild correlations or no significant correlations in almost all parameters. All parameters showed mild significant correlations in the total group but the stronger correlation was observed between MD and horizontal rim width (0.430). In general, correlations between structural parameters and global perimetric indices were better with MD than with CPSD.

Conclusion The correlations observed between optic disc parameters and SAP indices support the use of the optic nerve head OCT analysis software for glaucoma diagnosis.

■ 426

Diagnostic ability of the optic head measurements performed with the OCT in pre-perimetric glaucomas

MAYORAL F, POLO V, LARROSA JM, FERRERAS A, PLIEYO V, HONRUBIA FM
Ophthalmology Dpt, Miguel Servet University Hospital, Zaragoza

Purpose To evaluate diagnostic accuracy of the optic nerve head measurements performed with the optical coherence tomography (OCT) in pre-perimetric glaucomas.

Methods 98 normal eyes and 2 different pre-perimetric glaucoma subset of patients were included. Pre-perimetric glaucoma subjects presented normal standard automated perimetry and one of the following conditions: optic nerve head evaluated by means of stereo-photographs compatible with glaucoma (69 eyes) or abnormal short-wavelength automated perimetry –SWAP- (21 eyes). Only one eye was randomly chosen for the study. All of them underwent full ophthalmic exploration and an OCT (Stratus OCT 3000) evaluation of the optic nerve head. OCT results were compared between normal and pre-perimetric glaucoma groups. ROC curves were plotted to assess the diagnostic ability of the OCT parameters.

Results Significant differences ($p<0.05$) were found in all OCT parameters between normal and pre-perimetric glaucomas with glaucomatous optic disc. However pre-perimetric glaucomas defined by SWAP did not show differences in the OCT parameters respect to normal group. The greater area under the ROC curve was observed for the cup area.

Conclusion Optic nerve head parameters obtained by means of OCT can detect pre-perimetric glaucomas with structural damage, but not those with functional defects. Cup area showed the best sensitivity-specificity balance.

■ 427

Evaluation of structural parameters for the diagnosis of preperimetric glaucoma

GUERRI MONCLUS N, EGEA MC, PEREZ S, PABLO LE, BARAIBAR B, HONRUBIA FM
Ophthalmology Dpt, Miguel Servet University Hospital, Zaragoza

Purpose To compare the diagnostic ability between the quantitative parameters of two tomographers-OCT and HRTII- for the detection of preperimetric glaucoma

Methods A total of 150 suspect-glaucoma subjects were included in this prospective, clinical study. All of them were followed for eight years, eyes with perimetric changes were excluded from the study. A structural analysis on optic nerve by means of stereoscopic photography, OCT and HRTII was performed. Retinal nerve fiber layer red-free digital photographs were used to detect preperimetric glaucomatous changes.

Results HRT RB function showed the best capacity to spot those eyes with RNFL defects among the suspect group. Comparison between OCT and HRT best parameters were slightly better for the later in preperimetric glaucoma diagnosis.

Conclusion Both tomographers showed a limited capacity of preperimetric glaucoma diagnosis regarding their quantitative parameters.

■ 428

Effectiveness of long term follow-up by means of structural and functional test in early glaucoma diagnosis

EGEA MC, GUERRI N, PABLO LE, PEREZ S, SANCHEZ A, HONRUBIA FM
Dept of Ophthalmology, Hospital Universitario Miguel Servet, Zaragoza

Purpose To evaluate the long-term follow-up in a group of control, glaucoma-suspects and early glaucoma subjects using morphological and structural tests.

Methods A total of 251 consecutive subjects were included in this prospective, clinical study and followed for eight years. All of them underwent automated white on white perimetry, nerve head stereoscopic morphological study and retinal nerve fiber layer red-free photographic evaluation.

Results RNFL defects were significant higher in early glaucoma and suspect-groups vs control eyes. Vertical cup/disc cupping did not show differences among study groups. Regarding glaucomatous evolution RNFL defects proved to be more predictive than initial IOP over 25 or vertical cup disc over 0.7 .

Conclusion Long term follow-up in glaucoma suspect eyes, and their inclusion in different risk groups, allows an earlier glaucoma diagnosis.

■ 429

Diagnostic ability of the automated classification of the HRT3 versus the Moorfields regression analysis

FERRERAS A (1), PAJARIN AB (2), LARROSA JM (1), POLO V (1), MAYORAL F (1), PUEYO V (1), HONRUBIA FM (1)

(1) Ophthalmology, Miguel Servet University Hospital, Zaragoza
 (2) Primary care, Zaragoza

Purpose To compare the diagnostic performance of the contour-line independent Glaucoma Probability Score (GPS), with the results of the Moorfields Regression Analysis (MRA).

Methods 63 normal eyes and 73 glaucoma subjects (altered standard automated perimetry and glaucomatous optic nerve head appearance) were included. All of them underwent an optic disc tomography by using the Heidelberg Retina Tomograph (HRT3). The receiver operating characteristic curves were plotted for MRA and GPS in different subsets of disc area sizes.

Results No significant differences ($p<0.05$) were observed for age, disc area, and pachymetry between normal and glaucoma groups. In the total group, areas under the ROC curve (AUCs) were 0.823 for the MRA and 0.818 for the GPS. No significant differences were found between them. Sensitivity and specificity were 71.4% and 85.7% for the MRA, and 64.3% and 88.6% for the GPS respectively (cut-off point: outside normal limits, for both). No significant differences were observed between the AUCs of the MRA and the GPS in different disc area matched subgroups. Disc area of less than 1.6 mm² showed the lowest AUCs (0.758 for the MRA and .750 for the GPS).

Conclusion MRA and GPS showed similar diagnostic ability. When smaller is the disc area size, lower is the sensitivity-specificity balance.

■ 430 / 4156

Impact of Neuroretinal Rim Measurements as Predictive Factor for the Development of Glaucoma in Patients with Ocular Hypertension

LAEMMER R (1), MARTUS P (2), MARDIN CY (1)

(1) Department of Ophthalmology, University of Erlangen-Nuremberg, Erlangen
 (2) Institute of Biostatistics and Clinical Epidemiology, Charité University Medicine, Berlin

Purpose The purpose of this study was to investigate morphologic predictive factors for the development of glaucoma in patients with ocular hypertension (OHT).

Methods Two hundred eighteen eyes of 109 patients with OHT (normal visual field, normal optic disc appearance, IOP > 21 mmHg) and an observation period of more than 5 years were included in the study. All patients received an annual, detailed, standardized glaucoma examination. 21 eyes of 17 patients had marked neuroretinal rim loss and development of glaucomatous optic disc atrophy during follow up, identified by masked comparative evaluation of stereographic optic disc slides by two experienced observers independently from each other. 13 eyes of 10 patients from this subgroup developed reproducible visual field defects. Standard HRT-parameter (global and 4 sectors) from baseline examination were compared between stable and progressive patients.

Results Significant differences ($p<0.01$, Mann-Whitney-U test) between both groups were found for most of the investigated parameters. In the progressive OHT group rim area and rim volume were significant lower. Also mean and peak height contour were significant lower in the progressive OHT group. Results were similar for global data as well as for sector analysis.

Conclusion Patients with OHT and conversion to early glaucoma during follow up had already at baseline a lower neuroretinal rim area and rim volume, detected by scanning laser tomography, if compared with the non-progressive group. This supports recent studies showing that low neuroretinal rim area and volume in healthy discs maybe a risk factor for the development of glaucoma.

■ 431 / 4157

Inadequate anterior segment compensation is not a major cause of false-negative GDx Nerve Fiber Analyzer test results

MUSKENS RPHM (1, 2), HEEG GP (1), JANSONILIS NM (1, 2)

(1) University Medical Center Groningen, University of Groningen, Groningen

(2) Department of Epidemiology & Biostatistics, Erasmus Medical Center, Rotterdam

Purpose To investigate the influence of inadequate anterior segment compensation on the screening performance of the GDx Nerve Fiber Analyzer with fixed anterior segment compensation(GDx).

Methods The GDx-parameter Ellipse Average was measured in the macula as a measure of the inadequacy of anterior segment compensation in 29 false-negative glaucoma cases (i.e., glaucoma patients with a normal GDx test result, defined as the Number <30) and 105 controls.

Results Macula Ellipse Average was $57 \pm 11 \mu\text{m}$ (mean \pm SD) in the false-negative glaucomapatiens and $62 \pm 13 \mu\text{m}$ in the controls (t-test: $P=0.06$).

Conclusion Inadequate anterior segment compensation does not seem to be the majorcause of false-negative GDx test results.

■ 432

Agreement among Optical coherence tomography (OCT), Scanning laser tomography (HRT-II) and Scanning laser polarimetry (GDx) in open-angle glaucoma

PUEYO V, POLO V, LARROSA JM, FERRERAS A, ALIAS EG, HONRUBIA FM
 Ophthalmology, Zaragoza

Purpose To evaluate agreement among Scanning laser tomography (HRT-II), Optical coherence tomography (OCT) and Scanning laser polarimetry (GDx VCC) in open-angle glaucoma diagnosis.

Methods A total of 139 eyes from 139 subjects were included in the study and classified into healthy or glaucomatous depending on the intraocular pressure levels, optic nerve morphology and standard automated perimetry. All of them underwent an examination by means of HRT, OCT and GDx VCC, and several diagnostic criteria for glaucoma were defined from the structural measurements. Subjects correctly classified by these criteria were calculated and Venn diagram including the best criterion from each instrument was plotted.

Results Sixty-four out of the 139 subjects were classified as healthy and 73 as glaucomatous. The best criteria for glaucoma diagnosis were: FSM function < 0 (from HRT-II), at least one quadrant with RNFL thickness < 5% (from OCT) and NFI > 30 (from GDx VCC). By means of these criteria 69 out of the 73 glaucomatous subjects were correctly classified by any of the devices, being 39 classified as glaucomatous by the three of them. Scanning laser tomography was the instrument that detected glaucoma patients the best (61 out of 73).

Conclusion Some criteria defined by structural measurements provided by HRT-II, OCT and GDx VCC are useful in glaucoma diagnosis, showing high agreement among them.

■ 433

Influence of the corneal thickness on the measures of the no contact tonometry

MUNOZ MA (1), GONZALEZ F (2), PUELL MC (3), SANZ JC (3)

(1) *Human Anatomy and Embriology I, Madrid*(2) *Univeritary Clinics of Optometry, Madrid*(3) *Optics II (Optometry and Vision), Madrid*

Purpose The measure of the intraocular pressure (IOP) is fundamental in the clinical practice and it is used to carry out decisive diagnoses in the valuation and treatment of diverse and important pathologies. Some studies have been carried out to discover factors ocular that could act like sources of error in the determination of the value of the IOP. The corneal thickness has been that influences in the reading of the IOP. For this reason, we have analyzed in this work the values of the IOP obtained by means of no contact tonometry.

Methods The sample formed a group of 127 students of Optometry of the University School of Optics and Optometry from Madrid with a stocking of age of 20 years. The IOP was determined by means of a pneumotonometer of Nidek while the corneal thickness was determined by means of the Pachimeter of Tomey. The measures of IOP always took in the same horary in order to minimize the effects of the day variation of the IOP.

Results The IOP of the sample was from 13.40 mmHg. Showing a normal distribution and without significant differences for gender. The corneal thickness was from 544.10 micrometers. In the same way, the distribution is normal and it doesn't exist differences significant for sexes. The lineal adjustment between the values of IOP and the corneal thickness show dependence significant, whose coefficient is of 0.362. This means that the corneal thickness explains a 13.08% of the variability of the IOP.

Conclusion It is clear that in the pursuit of determining pathologies that show with an increase of the IOP, the measure of the intraocular pressure by itself could not assure us a reliable diagnosis. It is necessary to accompany it with the measure of the values of the corneal thickness.

■ 434

Importance of the central corneal thickness in the study of ocular hypertensive and pre-perimetric glaucomas

ALIAS EG, FERRERAS A, POLO V, LARROSA JM, PUEYO V, HONRUBIA FM

Dept of Ophthalmology, Hospital Universitario Miguel Servet, Zaragoza

Purpose To compare the results obtained with ultrasound pachymetry in normal, ocular hypertensive and glaucomatous subjects in different age groups.

Methods 254 eyes of 254 subjects were included. This sample was divided in 61 normal eyes (intraocular pressure -IOP- less than 21 mmHg, normal optic nerve head morphology and normal standard automated perimetry -SAP-), 193 eyes with ocular hypertension -OHT- (IOP>21 mmHg and normal SAP) and two groups of pre-perimetric glaucomas defined by IOP>21 mmHg, normal SAP and some of the following criteria: optic nerve head appearance compatible with glaucoma, evaluated by means of estereo-photographs (62 eyes) or abnormal short-wavelength automated perimetries (36 eyes). All groups were age matched and all of them underwent a corneal ultrasound pachymetry. The results of the main central corneal thickness (CCT) were compared between the normal group, and the OHT and pre-perimetric glaucoma groups in different sub-groups of age.

Results Significant differences ($p<0.05$) were observed between the normal and OHT group. CCT was thinner in the normal group than in the OHT group. When the ECCs between normal and OHT groups were compared by age groups, only were found differences in the 50 to 60 years range. Pre-perimetric glaucoma group did not present differences in the CCT with the normal group in any age sub-group.

Conclusion OHT group showed thicker corneas that contribute to overestimate the real IOP value. A thin CCT cannot be considered as a risk factor for developing glaucomatous damage.

■ 435 / 4155

Influence of Central Corneal Thickness in pre-perimetric glaucoma subjects defined by means of HRT II, OCT and GDx VCCBORQUE E, LARROSA JM, POLO V, FERRERAS A, MAYORAL F, HONRUBIA FM
Ophthalmology Dpt, Miguel Servet University Hospital, Zaragoza

Purpose To compare the corneal ultrasonic pachymetry results between normal and pre-perimetric glaucoma subjects in different age groups.

Methods 61 normal eyes (IOP<21mmHg, with normal standard automated perimetry -SAP- and no glaucomatous appearance of the optic nerve head) and three different pre-perimetric glaucoma subset of patients, were included. Pre- perimetric glaucoma groups were age matched with normal group, and presented IOP>21, normal SAP and one of the following criteria: MRA of the HRT II "borderline" or "outside normal limits" (53 eyes), average thickness of the OCT with $p<0.05$ (27 eyes) or NFI of the GDx VCC >29 (23 eyes). Only one eye was randomly chosen for the study. All of them underwent corneal ultrasonic pachymetry (DGH 500). Mean central corneal thickness (CCT) were compared between normal and pre-perimetric glaucoma subjects in different age groups.

Results In general terms, no significant differences were found in the CCT between healthy subjects and the pre-perimetric glaucoma eyes defined by the three structural tests in any of the age groups. No lineal relation was found between IOP and CCT.

Conclusion There is not a greater risk to develop glaucomatous damage in subjects with thinner CCT. It is complex to adjust IOP depending on CCT as the relationship between them is not lineal.

■ 436

Clinical comparison between rebound tonometry and Goldmann Applanation tonometry in patient with glaucoma's diagnosis.

RECHICHI MR, PESCE GP, FRATTO MF, CATALANO CC,

MESIANI MAZZACUVA FMM

Magna Gracia University, Catanzaro

Purpose The aim of this study is to evaluate the accuracy of ICare® in comparison with the Goldmann applanation tonometer (GAT) considering also the role of central corneal thickness.

Methods We compared IOP measurements in 124 eyes of 65 patients in which central corneal thickness was measured by A-Scan biometer. A unique operator performed first ICare® rebound tonometry and then, after topical corneal anesthesia, a Goldmann applanation tonometry. Central corneal thickness was measured for all eyes

Results Correlation between two techniques, evaluated with Pearson's test, was very high ($r=0.769$, $p<0.0001$). ICare® IOP measurements gave these results: mean IOP 16.45 mm/Hg, range 10-28 mm/Hg, SD 3.29 mm/Hg, CI 95% 15.86 to 17.03 mm/Hg. GAT values was : mean IOP 16.66, range 10-27 mm/Hg, SD 2.93 mm/Hg, CI 95% 16.14 to 17.19 mm/Hg. Mean difference between these two techniques was only 0.21 mm/Hg, ($p<0.01$).

Conclusion We can consider ICare® rebound tonometry a valid alternative to the GAT tonometry and we appreciated usefulness of ICare® also in pediatric ambulatory in which this portable tonometer is well accepted by little patients. The rebound tonometry, similarly to the GAT, seems to be influenced by the corneal thickness.

■ 437 / 2257

Intraocular pressure measurement using near infrared spectroscopy

KAMPPETER BA (1), WITZENMANN T (2), BACKHAUS J (2), JONAS JB (1)

(1) Department of Ophthalmology, Faculty of Clinical Medicine Mannheim, Mannheim
 (2) University of applied sciences, Mannheim

Purpose Despite advanced modern technology measurement of intraocular pressure is still a challenge. All systems in use depend on a indirect principle of measurement, influenced by tissue parameters. A new non invasive system to measure the intraocular pressure based on near infrared spectroscopy might overcome these limitations, by offering a real direct, non contact and non invasive intraocular pressure reading. It is based on the specific molecule absorption of near infrared radiation which is characteristically for individual molecule configurations. This principle is now being used in the human eye by measuring water molecule oscillation in the anterior chamber to assess the intraocular pressure transcorneally.

Methods 226 eyes from 113 patients were included in this study. Due to the impairment of existing pressure measuring devices, three different measuring techniques as reference methods for average calculation were used. Following standard Goldmann applanation tonometry, corneal thickness was evaluated and Goldmann readings were adjusted according to Shah. In addition, Dynamic contour tonometry (Pascal Tonometer; SMT Swiss Mircotechnology AG, Zurich, Switzerland) was performed.

Results Intraocular pressure readings ranged between 8 and 52mmHg (mean: 17.2 ± 5.8 mmHg). Mean discrepancy between near infrared spectroscopy's pressure values and mean of standard techniques was 4.43 ± 3.04 mmHg (Coefficient of correlation: $r^2=0.95$, RMSECV=2.2)

Conclusion Near infrared spectroscopy showed to be applicable in a real life environment for intraocular pressure measurement. Considering the lack of an objective intraocular pressure measuring device, results of this study suggest a high correlation between near infrared spectroscopy and standard devices.

■ 438

Finite element model of the cornea for applanation tonometry

LJUBIMOVA DY

Department of Mechanics, KTH, Stockholm

Purpose Intraocular pressure (IOP) measurement is an important parameter in the diagnosis and monitoring of glaucoma. To estimate the IOP with Goldmann applanation tonometry (GAT), a pressure is applied on the cornea to form an applanated circular area of certain size. For normal human corneas the applanating and intraocular pressures are assumed to be equal. However, recently it has been shown that applanating pressure is not always equal to true IOP, and depends on corneal thickness, curvature and rigidity. Furthermore, different refractive surgeries modify the corneal dimensions and structures thus affecting the accuracy of routine IOP measurement by GAT.

Methods An axisymmetric finite element model of cornea was developed to investigate the dependence of the true IOP on the applanating pressure and corneal biomechanical properties. Cornea was considered as varying in the thickness composite shell, exhibiting orthotropic material behaviour. Parameter nonlinear numerical analysis was performed as internal and applanating pressures were combined to model the applanation tonometry.

Results The effects of variation in each corneal variable on IOP readings was studied. The model shows that GAT is significantly influenced by cornea biomechanical properties, thickness and curvature.

Conclusion The results show a good correlation with different published data, demonstrating that magnitude of error in Goldmann tonometry reading may be clinically significant in some patients.

■ 439

Effects of physical exercise on intraocular pressure after the instillation of latanoprost eye drops

CHATZIBALIS T (1), NTAMPOS K (1), KARAMPATAKIS V (1), ECONOMIDES P (2), GARYFALLOS A (3), NATSIS K (4)

(1) Laboratory of Experimental Ophthalmology, Aristotle University, Thessaloniki
 (2) Ophthalmology Clinic, AHEPA University Hospital, Aristotle University, Thessaloniki
 (3) Fourth Department of Internal Medicine, Aristotle University, Thessaloniki
 (4) Department of Anatomy, Aristotle University, Thessaloniki

Purpose To study the behavior of intraocular pressure (IOP) after the instillation of latanoprost eye drops and the performance of a physical aerobic exercise.

Methods 20 healthy individuals were included. The initial IOP was measured at 21:00h of the previous night, followed by the instillation of one drop of latanoprost 0.005% in their right eye. 12 hours later (on the peak of the latanoprost effect), the IOP of both eyes was measured again. The individuals performed a physical aerobic exercise of moderate intensity on a bicycle ergometer for about 10 minutes (at 60-80 watts) and the IOP was measured again.

Results The mean IOP of the right eyes before the latanoprost instillation was ($\text{mean} \pm \text{SD}$) 14.08 ± 1.78 mmHg and after 12 hours 11.10 ± 1.79 mmHg (statistically significant difference, $P<0.001$). After the completion of the physical exercise the mean IOP of the right eyes was 9.25 ± 1.90 mmHg ($P<0.001$). As regards the left eyes, mean IOP before the latanoprost instillation on the fellow eye was 14.35 ± 2.10 mmHg and after 12 hours 14.25 ± 1.66 mmHg (not a statistically significant difference, $P=0.733$). Post exercise, mean IOP of the left eyes was 12.13 ± 1.58 mmHg (statistically significant difference, $P<0.001$). There was no statistically significant difference ($P=0.094$) between the two eyes, as regards the magnitude of the IOP reduction following the physical exercise.

Conclusion The instillation of latanoprost does not exclude the IOP reduction caused by physical exercise.

■ 440

Differences in Central and Peripheral Tonometry with ICare® Rebound Tonometry as a Function of Age

FERNANDES P (1), GONZALEZ-MEIJOME JM (1), JORGE J (1), QUEIROS A (1), ALMEIDA JB (1), PARAFITA MA (2)

(1) Physics-optometry, University of Minho, Braga
 (2) Optics-optometry, University of Santiago de Compostela, Santiago de Compostela

Purpose To evaluate the influence of age on the measurements and relationships among central and peripheral IOP readings taken with a rebound tonometer

Methods Three repeated measurements of intraocular pressure were taken with the ICare® rebound tonometer on the right eye of two-hundred and seventeen patients (88 males, 129 females), aged 18 to 85 years ($\text{mean} \pm \text{SD}$, 45.9 ± 19.8 years), at the center and 2 mm from the nasal and temporal limbus along the horizontal meridian. Three age groups were established as being less than 30 years old ($n=75$), from 31 to 60 years old ($n=77$) and above 60 years old ($n=65$).

Results There was a high correlation between central and peripheral IOP readings, with central reading being higher than peripheral ones, despite lack of statistical significance. The higher IOP values were found within the younger group for the central location. Subjects within the older group (above 60 years of age) presented significantly lower temporal IOP readings than the remaining two groups ($p<0.001$), while no significant differences were found among groups for central and nasal IOP readings ($p=0.099$ and $p=0.225$, respectively). There was a significant decrease in nasal and temporal IOP readings as the age increases ($p=0.011$ and $p=0.006$, respectively), what was not the case for central measurement ($p=0.059$).

Conclusion Peripheral rebound tonometry reflects a behavior as a function of age that central readings did not. Older patients have significantly lower values than the middle-aged and older patients in the temporal peripheral location. There is a trend towards a significant decrease in peripheral IOP as a function of age while central readings did not display such a trend.

■ 441

Analysis of Optineurin gene in Italian Normal Tension Glaucoma

FREZZOTTI P (1), LONGO I (2), CAPOROSSA A (1), MITTICA V (1), MELONI I (2), HADJISTILIANOU T (1), LOMURNO L (1), MOTOLESE P (1), RENIERI A (2)
 (1) Ophthalmology and Neurosurgery, Siena
 (2) Biologia Molecolare, Siena

Purpose To assess the influence of Optineurin gene in Normal Tension Glaucoma (NTG).

Methods 46 individuals (38 probands) were enrolled into the study, 31 of them being sporadic and the remaining belonging to 7 familial cases. Optineurin gene was analyzed in each index case using Denaturing High Performance Liquid Chromatography (DHPLC).

Results We detected one intronic deletion (IVS5 –28delGTT). Analysis of mRNA is ongoing to define the pathogenic role of the deletion. We didn't detect any other pathogenic mutation, while a number of polymorphisms (c.412G>A, c.IVS6-5C>T, c.IVS8-53T>C, c.IVS11-86G>T, c.IVS15-48C>A), including p.M98K variant were identified.

Conclusion These data suggest that the Optineurin gene is not a major gene for NTG.

■ 442

Bimatoprost, Latanoprost and Travoprost for the treatment of glaucoma: a cost-effectiveness analysis in Scandinavia

STEWART JA (1), STEWART WC (1), MYCHASKIW MA (2)
 (1) Pharmaceutical Research Network, LLC, Charleston
 (2) Pfizer, Inc., New York

Purpose To assess the cost-effectiveness of bimatoprost, latanoprost and travoprost in patients with open-angle glaucoma in Denmark, Norway, and Sweden.

Methods Cost-effectiveness analysis was performed using a Markov decision-analytic health economic model with stable and progressed glaucoma as the health states. Transition probabilities for open-angle and exfoliation glaucoma were derived from published medical literature and information regarding clinical practice patterns was obtained from surveys completed by 45 ophthalmologists dispersed throughout each of the countries. Country-specific unit costs were used for medications, clinic visits, diagnostics and outpatient services. Quality of life weights for various levels of visual acuity ranged from 0.50 to 0.68 and the effectiveness metric was the quality-adjusted life year (QALY). A 5-year time horizon was adopted, analyses were from a payer perspective and costs were discounted at 3% per year.

Results Effectiveness (years till progression) was within a narrow range (3.21 to 3.26 QALYs) across all products in each country. Latanoprost was 3% less expensive than bimatoprost and travoprost in Norway and Sweden and the costs of the 3 agents were within 1% of each other in Denmark. Latanoprost dominated (i.e., was more effective and less expensive than) bimatoprost and travoprost in Norway and Sweden. In Denmark, bimatoprost dominated travoprost. Although bimatoprost was slightly less expensive than latanoprost in Denmark (28,700 vs 29,000 DKK, respectively), latanoprost was more effective yielding an incremental cost-effectiveness ratio of 47,871 DKK.

Conclusion Latanoprost was more cost-effective than other available prostaglandin analogues.

■ 443

Latanoprost versus Timolol monotherapy for the treatment of glaucoma: a cost-effectiveness analysis in scandinavia and the united kingdom using a decision-analytic health economic model

MYCHASKIW MA (1), STEWART WC (2), STEWART JA (2)
 (1) Pfizer, Inc., New York
 (2) Pharmaceutical Research Network, LLC, Charleston

Purpose To assess the relative cost-effectiveness of monotherapy with latanoprost or timolol in the treatment of open-angle glaucoma in Denmark, Norway, Sweden (Scandinavia), and the United Kingdom (UK).

Methods Cost-effectiveness analysis was performed using a Markov model. The health states were stable and progressed glaucoma. Transition probabilities for primary open-angle and exfoliation glaucoma were derived from the medical literature and data concerning practice patterns were obtained from surveys completed by 54 ophthalmologists geographically dispersed throughout each of the countries. Country-specific unit costs were assigned for medications, patient visits, diagnostics, and therapeutic procedures. Quality of life weights for various levels of visual acuity ranged from 0.50 to 0.68. A payer perspective with a 5-year time horizon was adopted and costs were discounted at 3% for Scandinavia or 3.5% for the UK per year.

Results Latanoprost was less expensive than timolol, ranging from 5.4% to 6.7% less in Scandinavia and by 2.1% less in the UK. The range of effectiveness (years to progression of glaucoma) between treatment cohorts was narrow, from 0.003 to 0.01, which may have reflected the fact that the design assumed that physicians control most patients' glaucoma over 5 years by adding or changing therapy. Incremental cost-effectiveness ratios for latanoprost versus timolol were 447,857 DKK in Denmark, 457,212 NOK in Norway, 1,251,126 SEK in Sweden, and 6,087 GBP in the UK.

Conclusion Over a 5-year period, latanoprost monotherapy is as cost-effective as traditional timolol generics over a 5-year period in Scandinavia and the UK.

■ 444

When is it cost-effective to treat ocular hypertension? Results of a decision-analytic health economic model

STEWART WC (1), STEWART JA (1), MYCHASKIW MA (2)
 (1) Pharmaceutical Research Network, LLC, Charleston
 (2) Pfizer, Inc., New York

Purpose To assess the cost-effectiveness of treating ocular hypertension (OHT) in the United States.

Methods Cost-effectiveness was estimated using a Markov model. Health states were stable and progressed OHT. Data from the Ocular Hypertension Treatment Study (OHTS) were used to derive practice patterns and transition probabilities. Data were obtained from Blue Cross/Blue Shield for specific unit costs for medications, patient visits, diagnostics, and therapeutic procedures. A payer perspective was adopted, the time horizon was 5 years, and costs were discounted at 3% per year.

Results Across all OHT patients, the incremental cost-effectiveness ratio (ICER) was \$89,072 to prevent 1 case from progressing to primary open-angle glaucoma. After adjusting for risk factors for progression identified in multivariate analysis in the OHTS trial, minimally cost-effective ICERs were: 20 years above the mean age of 56 years, ICER = \$45,155; 4 mm Hg above the mean intraocular pressure of 25 mm Hg, ICER = \$46,748; 40 microns less than the mean central corneal thickness of 573 microns, ICER = \$36,683; and 0.2 wider than the mean vertical cup/disc ratio of 0.4, ICER = \$35,633.

Conclusion This Markov model was based on the results and practice patterns of the OHTS trial, and the results suggest that treating all OHT patients may not be cost effective. However, treating OHT patients with risk factors for progression, i.e., advancing age, higher intraocular pressures, thinner central corneal thicknesses, and wider vertical cup/disc ratios, does appear to be cost-effective in preventing the onset of glaucomatous damage.

■ 445 / 3425

Choroidal blood flow of patients undergoing an hemodilution for retinal vein occlusion

CHIQUET C (1), ROMANET JP (1), SAVY O (1), KUCHLER F (2), DRILLAT P (3), VINH V (1), TONINI M (1), GEISER MH (4, 2)

(1) Department of Ophthalmology, Grenoble

(2) Institut de Recherche en Ophtalmologie, Sion

(3) Etablissement Français du Sang, Grenoble

(4) Haute Ecole Valaisanne, Sion

Purpose To evaluate the effect of hemodilution on sub-foveal choroidal blood flow (ChBF) in the human eye with retinal vein occlusion

Methods ChBF was measured by laser Doppler flowmetry (LDF) in 16 patients (54 ±13 years, SD, between 22 and 81 years old) with retinal vein occlusion (RVO) in one eye. Isovolemic hemodilution was performed in patients with RVO after informed consent, when hematocrit was higher than 35%. ChBF was measured on both eyes 1 day before hemodilution, 1 one hour before each hemodilution and 1 hour later. Criterion to validate the measurement is the reproducibility of the amount of backscattered light from the fundus which is about three order of magnitude higher than the Doppler signal. LDF parameters were the flow (ChBF), the velocity (ChBVel) and the volume (ChBVol).

Results After hemodilution, hematocrit was reduced by 22% ($p<0.0001$). In healthy eyes ($n = 22$), hemodilution leads to an increase of ChBVel (7%) and ChBF (7%). In eyes with RVO ($n = 16$), the reduction of hematocrit was associated with a reduction of ChBVel (5%) and ChBF (6%). In both eyes, ChBVol did not change significantly. Comparison of the changes for each patient between affected eyes and contralateral healthy eyes was significant for ChBVel and ChBF.

Conclusion After isovolemic hemodilution, a significant decrease of hematocrit induced an opposite change of ChBF and ChBVel between affected eyes and contralateral healthy eyes. These preliminary results must be confirmed with a larger series of patients. Furthermore changes of choroidal blood flow parameters may not reflect changes in the retinal circulation.

■ 446 / 3426

The effects of intravenous histamine and histamine-receptor blockade on ocular blood flow

KARL K (1), RESCH H (1), WEIGERT G (1, 2), ZAWINKA C (3), GARHOFER G (1, 2), SCHMETTERER L (1, 4)

(1) Clinical Pharmacology, Vienna

(2) Ophthalmology, Vienna

(3) University Eye Clinic, Basel

(4) Biomedical Engineering and Medical Physics, Vienna

Purpose To investigate the effect of intravenous administered histamine on ocular blood flow and to identify the receptors mediating the histamine induced blood flow response.

Methods In study A placebo or histamine was administrated intravenously. In studies B and C histamine was infused in the absence or presence of the H1-receptor-antagonist diphenhydramine or the H2-receptor-antagonist cimetidine, respectively. In all three studies, choroidal blood flow was assessed with laser Doppler flowmetry. Retinal blood flow was calculated based on measurements of retinal vessel diameters using a retinal vessel analyzer and retinal blood cell velocity assessed with laser Doppler velocimetry.

Results Study A: Histamine significantly increased retinal arterial and venous diameters, whereas retinal blood flow was not affected. Choroidal blood flow increased after administration of histamine. Studies B and C: Co-administration of cimetidine did not modify the effects of histamine on ocular blood flow. In contrast, co-administration of diphenhydramine significantly reduced histamine-induced blood flow effects.

Conclusion Histamine induces an increase in choroidal blood flow as well as a vasodilatation in retinal arteries and veins. This effect can be blunted by co-administration of a H1 receptor antagonist. Our results strongly suggest that in the human retina and choroid histamine induced vasodilator effects are mediated by H1-receptors.

■ 447 / 3427

Effects of Indomethacin on Retinal and Choroidal Blood Flow in Healthy Volunteers

BERISHA F (1, 2, 3), WEIGERT G (1, 4), FUCHSJÄGER-MAYRL G (1, 4)

SCHMETTERER L (1, 5), GARHOFER G (1, 4)

(1) Department of Clinical Pharmacology, Vienna

(2) Schepens Retina Associates Foundation, Boston

(3) Ophthalmology, Harvard Medical School, Boston

(4) Department of Ophthalmology, Vienna

(5) Center for Biomedical Engineering & Physics, Vienna

Purpose Prostaglandins are assumed to play a role in ocular blood flow regulation. Animal studies suggest that retinal and choroidal blood flow decrease after administration of indomethacin, a nonspecific cyclooxygenase inhibitor. The aim of this study was to investigate the effects of indomethacin on ocular blood flow in healthy humans.

Methods Ten healthy male volunteers were studied in a randomized double-masked, placebo-controlled, two-way crossover design. Indomethacin or placebo was administered intravenously as a bolus (0.4 mg/kg) followed by continuous infusion of 0.4 mg/kg/h over 2 hours in two different study days. Ocular hemodynamics were measured at baseline, 30 minutes, 1, 2 and 3 hours after the start of the infusion. Retinal vessel diameters were assessed using a retinal vessel analyzer; retinal blood velocity was measured using the bidirectional laser Doppler velocimeter. Retinal blood flow was calculated based on retinal vessel diameter and red blood cell speed. Subfoveal choroidal blood flow was measured using laser Doppler flowmetry.

Results Indomethacin significantly decreased retinal blood velocity by -16±37% ($p=0.0359$ versus placebo) and retinal blood flow by -29±24% ($p<0.01$ versus placebo). Choroidal blood flow was also significantly decreased by -16±16% after indomethacin administration ($p=0.03$ versus placebo).

Conclusion Our results showed a marked decrease in retinal and choroidal blood flow, indicating that prostaglandins are involved in the regulation of ocular blood flow. The mechanisms underlying the decreasing effects of indomethacin on ocular blood flow remain to be clarified.

■ 448 / 4245

Adrenergic receptors in retinal arterioles

GISLADOTTIR S, EYSTEINSSON T, SIGURDSSON SB

Physiology, University of Iceland, Reykjavik

Purpose To examined the role of adrenergic receptors, in the smooth muscles of retinal arterioles, in the control of blood flow.

Methods Segments of retinal arterioles from bovine eyes were dissected out and placed in a small vessel myograph. The vessels were continuously bathed with a physiological saline solution (15 ml volume), at a temperature of 37°C and constant oxygen flow. Drugs were added to the bath accordingly (with minimum of n=5) and the contractile or dilative response recorded with the myograph (in mN).

Results The effect of the alpha and beta agonist noradrenaline (norepinephrine) was tested and evoked a significant contractile response. Beta-agonists gave no significant response (isoproterenol, an unspecific beta agonist and terbutalin, a specific beta₂ agonist). An unspecific alpha agonist (dihydroergotamine) and a specific alpha₁ agonist (cirazoline) elicited a significant contractile responses. A specific alpha₂ agonist (clonidine) elicited no significant response. Unspecific beta blockers (propranolol and timolol) significantly relaxed the contraction enhanced by noradrenaline, but only at high doses. The non-specific alpha blocker (phentolamine) significantly relaxed the contraction induced by noradrenaline and cirazoline.

Conclusion These results indicate that in the smooth muscles in bovine retinal arterioles, alpha₁ receptors are dominant. Noradrenaline and cirazoline are mediating their contractile effects through alpha₁ receptors whereas the alpha blocker phentolamine is blocking this response. It is also likely that beta receptors are not present and that the relaxation mediated by the beta blockers might be working through other mechanisms than the blockage of beta receptors, since they affected the noradrenaline response only at high doses.

■ 449 / 4246

Retrobulbar Use of Poloxamer for Ocular Drug Delivery

VEHANEN K (1), HORNOF M (2), URTTIA (2), LUUSITALO H (1)

(1) Department of Ophthalmology, University of Kuopio, Kuopio

(2) Viikki Drug Discovery Technology Center (DDTC), University of Helsinki, Helsinki

Purpose Use of injectable biopolymers for the controlled drug delivery will provide an option between the eye drops and surgically inserted implants. This study was undertaken to demonstrate the suitability of Poloxamer 407 (BASF) as a vehicle of controlled drug release as retrobulbar injections.

Methods Young Wistar rats were anaesthetised before retrobulbar injection of Poloxamer (25 % in 0.9 % NaCl with or without fluorescence markers). Control animals received retrobulbar injections of sodium hyaluronate. The release of fluorescence marker was detected by Fluorotron Master fluorophotometer (Ocumetrics). Rats were euthanised with CO₂ after 6, 12 and 24 hours, 3 and 7 days. Eyes were enucleated, embedded into paraffin and cut into 5 µm sections. Sections were stained for haematoxylin/eosin and immunostained for plasma fibronectin (CCBD), tenascin and inflammation marker (CD38) using 3,3'-diaminobenzidine as a chromogen. The analysis was done using light microscopy.

Results The gelation temperature of the 25 % Poloxamer formulation is 19°C and the gel dissolves in six hours *in vitro*. *In vivo* the gel containing FITC-Dextran is visualized parabolically near the site of injection six hours and very faintly 12 hours after the injection. FITC-Dextran was totally disappeared after 24 hours. Clinical evaluation of the eyes and histological analysis do not show pathological changes during the first week after injection.

Conclusion Retrobulbar injection of Poloxamer will give a release of compounds like FITC-Dextran for at least 12 hours. Based on the clinical and histopathological evaluation Poloxamer seem to be avoid of local toxicity. For clinical purposes a more prolonged dissolution time would be desired.

■ 450 / 4247

Structural Aspects of the Design of Ocular Drug Delivery SystemsMAHOMED A, TIGHE B¹¹Biomaterials Research Unit, Aston University, Birmingham

Purpose Use of a contact lens as an alternative vehicle to drops offers an opportunity to prolong the contact between the drug and cornea; thus offering a promising route for controlled ocular drug delivery. The potential of current soft lens materials were investigated for this application.

Methods Nelfilcon A, a PVA based lens material, is unusual since it is the only neutral daily disposable lens. This means that drug retention should be primarily dependent on the octanol/water partition coefficient ($\log K_{ow}$), values <0 = higher water solubility, >0 = higher octanol/lipid solubility, rather than on interaction of chemical groups. The $\log K_{ow}$ for a range of materials, drugs and drug models have been calculated and release kinetics from PVA and HEMA/NVP materials compared.

Results The $\log K_{ow}$ of lidocaine, a model ocular anaesthetic, is +2 when the drug is unionised. However drugs tend to ionise at physiological pH, thus the pH dependent octanol/water distribution coefficient ($\log D$) is probably a more valuable parameter. For example $\log D$ for lidocaine increases from -1 to +1.5 as pH increases from 4 to 8. In addition to the influence of $\log D$ to drug delivery, the release kinetics of a drug model from PVA and HEMA/NVP based lenses showed that the latter tended to interact strongly with the drug's acidic groups.

Conclusion The selection of an appropriate hydrogel-drug combination is a critical design aspect for controlled delivery. Macromer technology, e.g. nelfilcon A, which does not require a post-fabrication lens extraction process offers a practical manufacturing advantage. Release profiles are likely to be influenced by both octanol/water partition coefficients and specific chemical interactions. An appropriate "trigger" may be required to accelerate in-eye release.

■ 451 / 4345

Spatial properties and shortterm vasomotion of retinal vessels in vasospastic subjectsKOCHKOROVA A, GUGLETA K, ZAWINKA C, KATAMAYR R, FLAMMER J,
ORGIL S*Ophthalmology, Basel*

Purpose The purpose was to analyze spatial and temporal variations of retinal vessels in vasospastic subjects.

Methods Twenty-six women were divided in two equal groups, vasospastics and normal controls. Coefficient of variation (CV) of the vessel diameter was a measure of spatial irregularity. Coefficients of correlation (CC) of successive means and standard deviations of the vessel diameter within a defined time period was the measure of vessel diameter uniformity during vasomotion.

Results CV in arterioles: $8.8 \pm 2.8\%$ and $6.1 \pm 1.7\%$, CV in venules: $3.8 \pm 1.4\%$ and $3.6 \pm 0.9\%$ in vasospastics and normal controls, respectively (planned comparison: difference between groups in arterioles $p=0.007$). CC in controls: 0.11 ± 0.23 and 0.09 ± 0.23 , CC in vasospastics: 0.25 ± 0.40 and 0.24 ± 0.22 , in arterioles and venules respectively (difference between groups as one factor $p=0.038$, and difference between arterioles and venules as the other factor $p=0.77$, interaction $p=0.96$).

Conclusion Retinal arterioles in vasospastic subjects show higher spatial irregularity than normal controls. Both arterioles and venules demonstrate an altered vasomotion pattern in vasospastic subjects.

■ 452 / 4346

The effects of voluntary fasting on retinal and peripheral vessel reactivity – a case study

HEITMAR R, GHERGHEL D

School of Life & Health Sciences, Dept. Vision Sciences, Birmingham

Purpose To study the retinal and peripheral vessels reactivity, as well as the systemic blood pressure (BP) response to repeated voluntary fasting in a young and healthy subject.

Methods Measurements of the retinal vessels (one arteriole and one venule) reactivity to flicker (by means of the Retinal Vessel Analyser-RVA; IMEDOS, Jena, Germany) were undertaken in one healthy male subject aged 21 years after a 20-h period of voluntary fasting and then one hour after a meal; this experiment has been repeated on three separate occasions at the same time of the day. The RVA measurements consisted of 50 seconds baseline followed by three periods of 20 seconds flickering light provocation, each of them followed by 80 seconds observation. A mean value of the three separate measurements was calculated as the final dynamic vessel response. Systemic BP and peripheral blood flow (by means of capillary microscopy using the CAM1 Laser Doppler Capillary Anemometer, KK Technology, Devon, England) measurements were also performed on the second and third occasion.

Results The retinal vessels reactivity response was significantly lower in both the arteriole (mean dilation = $-0.17 \pm 0.55\%$) and venule (mean dilation = $+4.5 \pm 2.98\%$) under the fasting conditions comparing to that measured one hour after a meal (mean arteriole dilation = $+3.63 \pm 1.00\%$; and mean venule dilation = $+5.13 \pm 0.75\%$ respectively). These differences were statistically significant ($p<0.05$). There were no differences in either BP or capillary microscopy measurements between the two categories of conditions ($p>0.05$).

Conclusion The abnormal vascular reactivity induced by fasting was significant; however, the mechanism and implications of this observation need further investigation.

■ 453 / 4347

Optic Nerve Blood Flow and Retinal Diameter Responses to Flicker Stimulation are Described by a 2nd Order Linear System Model

RIVA CE (1), BONAIUTI M (2), ROVATI L (2)

(1) Oftalmologia, Università di Bologna, Bologna

(2) Dipartimento di Ingegneria dell'Informazione, Università di Modena e Reggio Emilia, Modena

Purpose Optic nerve blood flow (F) and retinal vessel diameter (D) increase in response to increased neural activity. We applied a control system analysis to describe the main dynamic features of this regulatory process.

Methods F was measured by LDF and D by RVA in healthy subjects (age 25-62 years). Neural activity was evoked by diffuse luminance flicker (various frequencies and 1-8 min duration). The F and D time-courses underwent a 10-point lagging running-averaging process and then were fitted with a modified 2nd-order control system G(s) consisting of the cascade of a Proportional-Derivative (PD) term, a 2nd-order Filter (F2) and an Integrator term (INT). The following characteristic parameters of G(s) were determined: the undamped natural frequency (ω) of F2, the damping factor (ξ) of F2, the gain (K) of PD, the rate time (Tv) of PD and the integrator time constant (r) of INT.

Results The flicker-induced increase in F during stimulation and the decrease in D after cessation of the stimulus could be well fitted by the response of G(s) to the Heaviside step function (correlation R²>0.66). Thus for the F-response, the following results were obtained: $\omega = 2.31 \pm 1.57$ (se) rad/sec, $\xi = 0.28 \pm 0.1$, K = 180.75±11.76, Tv = 2.67±1.62 sec, $\tau = 5.95 \pm 1.93$ sec. For the D-response the following values were obtained: $\omega = 0.14$ (se) rad/sec, $\xi = 0.071$, K = 0.01, Tv = 29934 sec, $\tau = 38.7$ sec (correlation factor R² = 0.98).

Conclusion The response of optic nerve blood flow and retinal vessel diameter to diffuse luminance flicker can be described by a second order linear system. The physiological basis of the various terms of the control cascade remains to be established.

■ 454 / 4444

Oximetry in retinal vascular occlusions

HARDARSON SH (1), KARLSSON RA (2), HALLDORSSON GH (2), JOELSSON SR (2), EYSTEINSSON T (1), BENEDIKTSSON JA (2), BEACH JM (2), STEFANSSON E (1)

(1) University of Iceland, Ophthalmology, Reykjavik

(2) University of Iceland, Electrical and Computer Engineering, Reykjavik

Purpose To analyze hemoglobin oxygen saturation (SatO₂) in retinal vascular occlusions using an automatic non-invasive oximeter.

Methods The oximeter is composed of a fundus camera, beam splitter and a digital camera. Specialized software analyzes images from the oximeter and calculates the SatO₂ within retinal vessels. Measurements were made on 13 patients with branch retinal vein occlusion (BRVO), 1 with hemivein occlusion, 2 with central retinal vein occlusion (CRVO) and 5 with branch arteriolar occlusion (BRAO).

Results In patients with retinal vein occlusions (RVO, all types) the venous SatO₂ in healthy eyes was 52±13% (mean±SD; n=12), 54±11% in non-affected venules of RVO eyes (n=12), 35±20% in affected venules before laser treatment (n=10) and 53±5% in affected venules after laser treatment (n=8). Before laser treatment, the affected venules had a significantly lower SatO₂ than the non-affected venules (p<0.01) or venules in the healthy eyes (p<0.05). In the 5 BRVO eyes measured both before and after laser treatment the venous SatO₂ rose from 45% to 53% (p=0.041). The SatO₂ in the occluded hemivein (1 case) increased stepwise following addition of laser treatment. Both CRVO eyes were very hypoxic. The SatO₂ was normal in the 5 BRAO eyes except for a hypoxic venule in the area of occlusion in 1 eye (the most recent BRAO).

Conclusion The oximeter shows decreased venous SatO₂ following RVO and a rise in SatO₂ with laser treatment.

■ 455 / 4445

Diabetes-induced cataract amplified in mice lacking CuZn-superoxide dismutase

OLOFSSON EM (1), MARKLUND SL (2), BEHNDIG A (1)

(1) Department of Clinical Sciences/Ophthalmology, Umeå University, Umeå

(2) Department of Medical Biosciences, Clinical Chemistry, Umeå University, Umeå

Purpose Lenses from mice lacking the antioxidant copper-zinc superoxide dismutase (SOD1 null mice) develop cataract and show signs of cell damage when exposed to high levels of glucose in vitro. The superoxide radical, elevated in the SOD1 null lenses, is thought to contribute to the cataractogenesis in these lenses possibly through the reaction with nitric oxide, generating the highly toxic peroxynitrite. In this study, we aimed to evaluate the effect of streptozotocin-induced diabetes mellitus on cataract formation in SOD1 null mice in vivo.

Methods SOD1 null and wild-type mice were made diabetic by repeated intraperitoneal streptozotocin injections for 5 consecutive days. Mice injected with citrate buffer were used as controls. After eight weeks of diabetes, the mice were sacrificed and the lenses removed and photographed in retro-illumination. The cataract formation was quantified from the photographs using digital image analysis.

Results After eight weeks, the diabetic SOD1 null mice showed more cataract compared to all the other groups (2-way ANOVA; diabetic*SOD1 null; p=0.001).

Conclusion This in vivo study supports our previous in vitro studies and gives further evidence on the protective role of SOD1 against diabetic cataract formation.

■ 456 / 4446

Transient Hypertonic Saline (ths)-induced Intraocular Pressure (IOP) as a rabbit model to assess potential new antiglaucoma drugs

BASTIA E, IMPAGNATIELLO F, BORGHI V, BIONDI S

Nicox Research Institute, Bresso, Milan

Purpose Reducing IOP is the major therapeutic strategy for the treatment of glaucoma. Indeed, various animal models have been described, even though their sensitivity to drug treatment differs considerably, making difficult the selection of new compounds. In this study, we describe the effects of different drugs acting on aqueous humor inflow such as timolol and dorzolamide in THS-induced IOP in NZW rabbits as well as in normotensive NZW and pigmented DB rabbits.

Methods In THS, IOP was raised by the injection of hypertonic saline (5%) into the anterior chamber.

Results Raised IOP reached a peak at 30 min (2-3 fold over basal, 16±2mmHg), stabilized during the following 60 min and decayed gradually in the following 3h. Topical application of timolol significantly and dose-dependently (▲maxIOP: -3+3; -6+3; -7+2mmHg, at 0.1, 0.3 and 1% respectively) reduced IOP increase, starting 30 min after instillation. Similarly, dorzolamide (2%) lowered IOP (▲maxIOP: -6+2mmHg) from 30 min after instillation. In DB rabbits, basal IOP was significantly higher compared to NZW (20 vs 16mmHg). Timolol (1%) and dorzolamide (2%) significantly lowered IOP at 60 min after instillation (▲maxIOP: -5+1; -7+1mmHg, respectively). Conversely, in normotensive NZW rabbits, both timolol and dorzolamide only slightly and not significantly reduced IOP.

Conclusion We show that the THS-induced IOP rabbit model is suitable to detect the IOP-lowering effect of commercially available drugs. Further studies are ongoing to evaluate the potential usefulness of this model for the screening of newly synthesized drugs.

■ 457 / 4447

Invasive Real Intraocular Pressure (IOP) Measurement. How to do it correctly

HERNANDEZ VERDEJO JL (1), ROMAN JM (2), BOLIVAR G (3),
FERRER B (2), TELUS MA (2)

(1) VISSUM Corporación oftalmológica., Madrid
(2) VISSUM, Madrid
(3) Universidad de Alcalá, Madrid

Purpose To show an experimental model to demonstrate the correct way to measure real IOP in porcine eyes.

Methods Values of real IOP was measured in freshly enucleated porcine eyes. Two reusable blood pressure transducers were used. The globes were inflated with 5% glucosated solution through the optic nerve to attain an IOP of 10-20 mmHg. A 27-gauge catheter was inserted from pars plana to the anterior chamber and another 27-gauge catheter was inserted into the vitreous cavity with any response, then the vitreous catheter was change for a 21-gauge catheter. Real IOP was then transmitted via the catheter liquid column to both external sensors. At that moment the IOP was increased into the anterior chamber via another catheter connected to a liquid column. IOP changes were registered for a period of 60 seconds.

Results Using the 27-gauge catheter, our model was unable to register changes of IOP in the vitreous chamber (VC). However, when the 21-gauge catheter was used, the transducers detected simultaneously changes of IOP both in the anterior chamber (AC) and in the VC until an IOP of 40 mmHg was reached. Beneath that point, there was a delay in the IOP raise into the VC, so when a level of 80 mmHg was reached into the AC, the transducer measured an IOP 20 mmHg less into the VC. At the end of the measurements IOP values remained stable.

Conclusion Our experimental model demonstrates that cannulation into the vitreous chamber as invasive method to measure IOP is not the correct way to register real IOP.

■ 458

Lipofuscin accumulation in the retina due to Zinc deficiency

KOZEIS N (1), KOKKINOU D (1, 2), FELEKIDIS A (1), SCHRAERMAYER U (2)

(1) Eye department Hippokrateion Hospital, Thessaloniki
(2) Department of VitreoRetinal Surgery, Tuebingen

Purpose To investigate if melanin in the ocular tissues of Long Evans (LE) and Wistar rats is affected, after a six months zinc free diet. Age-related macular degeneration (AMD) is less prevalent in humans with black fundus pigmentation, suggesting a possible correlation with melanin. Zinc, as an antioxidant, binds to melanin in pigmented tissues

Methods LE and Wistar rats were kept for over 6 months on a zinc-free diet (7 animals per group). The same quantity was used as a control. The animals were clinically examined by electroretinography (ERG). The retina and choroid of the animals were further investigated using light, fluorescence and electron microscopy and immunohistochemistry.

Results The ERGs indicated a greater impairment of the pigmented animals compared to less pigmented. Loss of photoreceptor nuclei was observed in both strains of zinc deficiency rats. Significant enhancement of lipofuscin accumulation, changes in shape and size of the melanosomes and infiltrating pigmented macrophages were detected in the zinc deficiency rats.

Conclusion Zinc deficiency animals were more damaged compared to controls. Our results indicated that melanin plays an important role in zinc metabolism. Zinc deficiency leads to lipofuscin accumulation in pigmented rats. There is an indication of a different mechanism of lipofuscin formation between pigmented and non pigmented animals. This should be under consideration when zinc is used in therapeutical trials of ocular diseases, e.g. AMD.

■ 459

Activities and concentrations of matrix metalloproteinase 9 and 2 (MMP-9 and MMP-2) in human diabetic aqueous humor

LEWANDOWSKA AB (1), KOMOROWSKI J (2), HRABEC E (3), GOS R (4), NOWAK MS (4)

(1) Dept. of Ophthalmology of the Medical University of Łódź, Tódz
(2) Endocrinology, Tódz
(3) Biochemistry, Tódz
(4) Ophthalmology, Tódz

Purpose Matrix metalloproteinases are a family of zinc containing enzymes that remodel extracellular matrix in health and disease. Proliferation of new vessels is associated with upregulation of metalloproteinase activities possibly via vascular endothelial growth factor in atherosclerotic plaques. We aimed to assess whether similar relationship exists in eye tissue. We assessed concentrations and activities of MMP-2 and MMP-9 in aqueous humor of patients suffering from diabetic retinopathy.

Methods 77 samples of aqueous from 77 eyes of patients undergoing routine cataract surgery were analysed. 69 of them suffered from diabetes and 8 served as a control group. 40 consecutive samples were analysed by ELISA assay, and 37 by gelatin zymography. Protein content of the samples were also analysed. Diabetic patients were divided into three groups; with minimal and no retinopathy, with maculopathy and with proliferative diabetic retinopathy. Statistica 6.0 was used for data analysis.

Results The levels of MMP-2 were found to be three-fold elevated in proliferative retinopathy group, mainly in the proenzyme form. MMP-9 levels were detected in 8 samples by ELISA assay and only in 2 samples by gelatin zymography.

Conclusion MMP-2 and MMP-9 are likely to be involved in the process of proliferative diabetic retinopathy. MMP-9 activity was present only in some of the proliferative retinopathy samples and further studies are needed to evaluate other factors responsible for their activation.

■ 460

Aqueous Humor Concentrations of Moxifloxacin, Gatifloxacin and Levofloxacin Following Topical Ocular Administration of VIGAMOX®, GATIFLO® or CRAVIT® Ophthalmic Solutions to Rabbits

IPINAZAR LINDURRAGA M (1), ROYA (2), SANDERS M (2), TRAWICK D (2), CURRY S (2), JASHEWAY D (2), COCKRUM P (2), DAHLIN D (2)

(1) Alcon, Barcelona
(2) Alcon Research, Ltd., Fort Worth, Tx

Purpose To evaluate ocular absorption of moxifloxacin (Moxi), gatifloxacin (Gati) and levofloxacin (Levo) following topical ocular administration of VIGAMOX®, GATIFLO® or CRAVIT® ophthalmic solutions by measuring the concentrations of Moxi, Gati and Levo in aqueous humor of New Zealand White (NZW) rabbits.

Methods Three groups of male NZW rabbits, at 15 minute intervals, were instilled three unilateral topical ocular doses (50 µL) of VIGAMOX®, GATIFLO® or CRAVIT® ophthalmic solutions into the cul-de-sacs of the right eye. Following the final instillation aqueous humor samples were collected from both eyes at specified time points up to 4 hours and drug concentrations were determined by reverse phase HPLC methods. Aqueous humor mean maximal concentrations (Cmax), time to Cmax (Tmax) and pharmacokinetic parameters were calculated by a one-compartment model.

Results All three drugs were absorbed into the eye and aqueous humor Cmax of Moxi was approximately 8- and 3-fold higher than that of Gati and Levo, respectively. Mean observed Cmax (model predicted Cmax) and model predicted Tmax values in aqueous humor were: Moxi 6.58 (5.67) µg/mL, 0.37 hr; Gati 0.796 (0.785) µg/mL, 0.85 hr; and Levo 2.33 (1.89) µg/mL, 1.03 hr.

Conclusion The results show that moxifloxacin was absorbed faster and provided substantially higher aqueous humor levels than gatifloxacin and levofloxacin.

■ 461

Long-term effect of dorzolamide/timolol unfixed combination on ocular blood flow

MARTINEZ-GARCIA A, SANCHEZ-SALORIO M
INGO, Santiago de Compostela

Purpose Purpose: to evaluate the long-term effect of dorzolamide 2%, twice daily (BID) on the retrobulbar hemodynamics in open-angle glaucoma patients.

Methods Methods: 40 eyes of 40 patients with documented open-angle glaucoma treated with timolol maleate 0.5% twice daily were included in this prospective longitudinal open-label study. Ocular hemodynamics were assessed by Color Doppler Imaging (CDI). The main outcomes of the study were peak systolic velocity (PSV), end-diastolic velocity (EDV) and Pourcelot Resistance index (RI) in the ophthalmic artery (OA), short posterior ciliary arteries (SPCA) and central retinal artery (CRA). Intraocular pressure (IOP) was tracked as observational parameter. Measurements were made in all patients at baseline, before dorzolamide 2% was started, and every 6 months during a 48-months follow-up period. Intralgroup comparisons between baseline and dorzolamide conditions were made using a Paired Student's t test. Statistical significance was set at p<0.01.

Results Results: The RI was significantly lower in the OA, SPCA and CRA, in all study visits, after dorzolamide was added, p<0.0001. The EDV was statistically significantly higher, p<0.0001, in the OA, SPCA and CRA, in all study visits, following dorzolamide application. After 4 years of treatment with dorzolamide and timolol maleate, PSV in the SPCA and CRA was significantly higher compared to baseline, p<0.001. Dorzolamide 2% reduced intraocular pressure (IOP) from 19.18 (1.34) to 18.07 (1.31) mm Hg, p=0.0001, with a mean follow-up of 45.30 (7.44) months.

Conclusion Conclusion: Dorzolamide 2% BID added to timolol maleate 0.5% showed a beneficial long-term effect on the retrobulbar hemodynamics in patients with open-angle glaucoma.

■ 462

Protective effects of ocular hypotensive drugs against the neuronal damage occurred by oxygen-induced retinopathy in the newborn rat

GUNGOR K (1), TUGLU IH (2), CEZAYIRLI E (3), VAROL T (3), OZBILGIN K (2), BEKIR NA (1)

(1) Dept of Ophthalmology, Gaziantep University Medical School, Gaziantep
(2) Dept of Histology and Embryology, Celal Bayar University, Medical School, Manisa
(3) Dept of Anatomy, Celal Bayar University, Medical School, Manisa

Purpose We aimed to determine whether bimatoprost, latanoprost and travoprost, ocular hypotensive drugs, has retinal neuroprotective effects in experimental in vitro and in vivo models.

Methods Oxygen-induced retinopathy (OIR) similar to that seen in premature infants can be produced in newborn rats by exposure to 80% oxygen for the first 5 days of life, with subsequent recovery for 7 days in a room air environment. The retinal damage was evaluated by counting the number of cells in the ganglion cell layer (GCL) by morphometry. Bimatoprost, latanoprost, and travoprost, were topically applied to eyes, and these were enucleated after various intervals. Eyes were removed and fixed in formaline for histology (H-E, PAS, Mason's Trichrome), immunohistochemistry (NOS, Laminin,TUNEL) and morphometry.

Results OIR in retinas from the ocular hypotensive drugs was less severe than that seen in untreated eyes. Protein expression of NOS, Laminin,TUNEL was increased in retinopathy which were decreased by drugs.

Conclusion These results suggest a potential neuroprotective effect of bimatoprost, latanoprost and travoprost, against retinal damage with different mechanisms on the cell behaviour.

■ 463

Effects of prostaglandins on Anterior Chamber Depth in Patients with Glaucoma or Ocular Hypertension.**A control-matchet study**

GUTIERREZ C, TEUS MA, BOLIVAR G
Hospital Universitario Príncipe de Asturias, Alcalá de Henares

Purpose Prostaglandins, might cause a decrease of the extracellular matrixes, such as collagen, and changes in fibrillin-1, which are components of the ciliary zonules. However, the mechanical effect of these changes on the dynamics of the ciliary zonules is unknown. This study evaluated the effect of latanoprost 0.005% on the anterior chamber depth (ACD) in patients with high intraocular pressure.

Methods We conducted a prospective, nonrandomized, autocomparative trial that included 40 patients (40 eyes) with glaucoma or ocular hypertension. The ACD was measured with ultrasonography before and after 1 month of treatment with latanoprost. To asses the reproducibility of the measurements a control group with 20 patients (20 eyes) who did not received latanoprost was used.

Results The mean ACD before treatment with latanoprost was 3.14 mm (standard deviation [SD], ±0.46) after 1 month of treatment with latanoprost, the ACD was 2.98 mm (SD, ±0.44). P≤0.05 for all comparisons

Conclusion These findings suggest that latanoprost decreases the ACD in patients with glaucoma or ocular hypertension after 1 month of treatment.

■ 464

Effects of acute increases of the intraocular pressure on the corneal pachymetry en eyes treated with travatoprost. An animal study

BOLIVAR G (1, 2), CASTEJON MA (1), TEUS MA (1, 2, 3)
(1) Hospital Universitario Príncipe de Asturias, Alcalá de Henares
(2) Universidad de Alcalá, Alcalá de Henares
(3) Instituto Ofalmológico Internacional VISSUM, Madrid

Purpose To observe if topical prostaglandin therapy modifies the corneal thickness and the effect of acute changes of the intraocular pressure on the corneal pachymetry in rabbit eyes.

Methods Prospective study with rabbits treated unilaterally with travatoprost for 1 month. The intraocular pressure in the anterior chamber is measured by direct cannulation and the pachymetry by an ultrasonic pachymeter under general anaesthesia. The basal corneal thickness is measured and also after setting the intraocular pressure (IOP) at 15 and 30mmHg.

Results We obtain statistically significant differences in the basal corneal thickness of both groups (p<0.01) and also in the decrease of corneal thickness that was observed after setting the IOP at 15mmHg (p=0.01) and at 30mmHg (p=0.02).

Conclusion The prostaglandin analogue therapy induces changes in the cornea of rabbits that imply differences in the corneal thickness at the basal level and also a different response to acute changes of intraocular pressure.

■ 465

Effects of physical aerobic exercise on intraocular pressure after instillation of timolol maleate eye drops

KARAMPATAKIS V (1), NATSIS K (2), ECONOMIDES P (3), CHATZIBALIS T (1), NTAMPOS K (1), STANGOS N (3), MERMOUD A (4)

(1) Laboratory of Experimental Ophthalmology, Aristotle University, Thessaloniki

(2) Laboratory of Anatomy, Aristotle University, Thessaloniki

(3) AHEPA Hospital, Eye Clinic, Aristotle University, Thessaloniki

(4) Hopital Ophtalmique Jules Gonin, Lausanne

Purpose To evaluate the influence of physical aerobic exercise on intraocular pressure (IOP) after the instillation of a non-selective b-adrenergic blocker (timolol maleate 0.5% eye drops).

Methods The IOP of both eyes was measured by applanation tonometry in 40 healthy individuals. Timolol maleate 0.5% eye drops were then instilled in the right eyes of the individuals and 2 hours later the IOP was measured again. Thereafter, the individuals performed a physical aerobic exercise on a bicycle ergometer for about 10 minutes at 60-80 Watts. IOP was measured again 5 minutes after the completion of the exercise. All measurements were performed from 8:00 to 11:00 a.m.

Results The mean IOP of the right eyes before the b-blocker instillation was (mean \pm SD) 15.75 ± 1.46 mmHg and two hours after 11.92 ± 1.37 mmHg (statistically significant difference, $P < 0.001$). After the completion of the exercise the mean IOP of the right eyes was 9.80 ± 1.36 mmHg ($P < 0.001$). As regards the left eyes, mean IOP before the b-blocker instillation on the fellow eye was 15.62 ± 1.37 mmHg and two hours after 13.90 ± 1.28 mmHg ($P < 0.001$). After the exercise, mean IOP of the left eyes was 11.65 ± 1.51 mmHg ($P < 0.001$). The mean IOP lowering effect of the exercise was 2.12 ± 0.72 mmHg for the right eyes and 2.25 ± 0.81 mmHg for the left eyes.

Conclusion After the b-blocker instillation in one eye, the IOP decreases also in the fellow eye. After a physical aerobic exercise the IOP still drops, regardless of the instillation of timolol maleate. Conclusively the non-selective b-adrenergic blockade does not exclude the exercise-induced IOP lowering effect.

■ 466

Diurnal variability of the ocular pulse amplitude (OPA) measured with Dynamic Contour Tonometry (DCT- Pascal Tonometry) in normal subjects

POLIRJAVAN S, DETRY-MOREL M

St. Luc University Hospital, UCL, Brussels

Purpose DCT measures the haemodynamic IOP fluctuations and the OPA. OPA reflects indirectly the choroidal perfusion and is therefore a possible independent risk factor in glaucoma. The characteristics of OPA are unclear. we wanted to assess OPA variability in normals.

Methods Prospective randomized study including 52 eyes of 28 normal subjects with GAT<22 mmHg. Exclusion criteria: intake of systemic medications which could interfere with blood pressure or heart rate. The IOP was measured at 9 am, 1 pm and 4 pm by Goldmann Applanation Tonometer (GAT) and DCT. 2 consecutive GAT followed by 3 consecutive measurements with DCT were performed on each session by the same clinician. Only DCT measurements with quality of 1 and 2 were taken into account. Blood pressure, pulse rate and pachymetry were recorded after the last IOP measurements. Spearman correlation coefficient were used to asses the correlation

Results The mean age was 40 ± 14 years. The mean GAT was 15.0 ± 3 mmHg. The individual amplitude (SD) of diurnal GAT fluctuations was 1.5 mmHg. There was a decreasing trend of -0.7 ± 0.1 mmHg between each measurement ($P < 0.0001$). The mean DCT IOP was 16.6 ± 2.8 mmHg. The individual amplitude of diurnal DCT IOP fluctuations was 2.1 mmHg. DCT IOP showed a decreasing trend as well by -0.5 ± 0.1 mmHg. The mean OPA was 2.2 ± 0.7 mmHg. The mean amplitude of diurnal OPA fluctuations was 0.4 mmHg. There was no evidence of a period effect ($P = 0.37$). The OPA was correlated with GAT: $r = 0.31$ $P < 0.0001$ as well as with IOP measurements obtained by DCT ($r = 0.49$ $P < 0.0001$). It wasn't correlated with blood pressure nor age.

Conclusion There is no evidence of diurnal trend for OPA in normals.

■ 467

Redox regulation of the 20S proteasome in human lens epithelial cells and intact mouse lens

PETERSEN A (1), CARLSSON T (1), KARLSSON JO (1), ZETTERBERG M (2, 1)

(1) Institute of Biomedicine, Department of Medical Chemistry and Cell Biology,
Göteborg

(2) Institute of Neuroscience and Physiology, Section of Ophthalmology, Mölndal

Purpose To study regulation of the 20S proteasome by redox-changes in the GSH/GSSG-system

Methods Human lens epithelial cell (HLEC) lysate was assayed for chymotrypsin-like, trypsin-like and peptidylglutamyl peptidase-like activity of the 20S proteasome using specific fluorogenic substrates. The assay was performed with/without addition of the reduced and the oxidized form of glutathione (0.2, 2 and 20 mM of GSH and GSSG respectively). Proteasome activity was also measured in HLEC lysate after addition of the reducing agent dithiotreitol (DTT) (2 and 20 mM) and after oxidative stress induced by incubation of cultured HLEC with 100 μ M H₂O₂. Intact mouse lenses were subjected to 100 μ M H₂O₂ for 7 days, prior to determination of GSH levels and chymotrypsin-like proteasome activity.

Results All three main enzyme activities of the proteasome in HLEC lysate were affected by GSH or GSSG addition. The chymotrypsin-like and peptidylglutamyl peptidase-like activities responded similarly with stimulation upon addition of GSH, whereas the trypsin-like activity was inhibited. Addition of GSSG caused inhibition of all three peptidase activities, but simultaneous incubation with DTT reversed the inhibitory effect of GSSG. Oxidative stress induced by H₂O₂ significantly decreased the main peptidase activities of the proteasome in cultured HLEC. Intact mouse lenses incubated with H₂O₂ showed trends of decreased chymotrypsin-like proteasome activity as well as decreased GSH-levels.

Conclusion The present data suggest that the intracellular GSH/GSSG ratio is important for regulation of the 20S proteasome and indicate a tight coupling between the redox system and the proteolytic machinery.

■ 468

Triamcinolone acetonide induces non apoptotic cell death of retinal pigment epithelial cells in vitro and in vivo

VALAMANESH F (1, 2), TORRIGLIA A (1), SAVOLDELLI M (3)

GANDOLPHE C (1), BEHAR-COHEN F (1, 2)

(1) INSERM U598, Paris

(2) Rothschild Ophthalmic Foundation, Paris

(3) Hôtel-Dieu Hospital, Paris

Purpose To study the potential toxicity of triamcinolone acetonide (TA) on retinal pigment epithelial cell line (ARPE-19) in vitro and in vivo.

Methods Sub confluent ARPE-19 cells were treated with 0.1 or 1 mg/ml Triamcinolone acetonide (TA). Cell viability was evaluated at 24 hours using MTT assay and Trypan blue test. Apoptosis was evaluated by TUNEL assay and annexin-binding. Immunohistochemistry was performed with anti-apoptosis inducing factor, cytochrome C, and LEI/L-Dnase II antibodies. Western-blots analysis was performed for activated caspase-3, phosphorylated JNK. Cytoplasmic vacuoles were labeled with monodansylcadaverine. For in vivo experiments, 50 μ g of TA were injected in the vitreous of Lewis rat eyes and ultra-thin analysis was performed 8 days later.

Results TA induced a dose-dependent RPE cell death. No sign of apoptosis were detected either by TUNEL assay, annexin binding, nuclei staining or activated-caspase 3 on western-blots. The alternative LEI/Dnase II pathway was not activated. Intense vacuolization positive for monodansyl cadaverine along with phosphorylation of JNK were detected. Transfection with Alix prevented TA-induced cell death, demonstrating that paraptosis was taking place. TEM images, most suggestive of paraptosis were also observed in RPE 8 days after TA injection in the rat eye.

Conclusion In vitro and in vivo observations demonstrate that TA induce a parapoptotic cell death in RPE cells, that may have clinical implications.

■ 469

Safety of high doses intravitreal triamcinolone: two years outcome

RUIZ MORENO JM (1, 2), MONTERO J (2), LLIGO F (2), AMAT P (2)
 (1) Department of Ophthalmology, Miguel Hernández University School of Medicine, Alicante
 (2) Instituto Ofthalmológico de Alicante, Vitreo-Retinal Unit, Alicante

Purpose To evaluate the safety of the intravitreous injection of high dose triamcinolone acetonide (TA).

Methods Prospective, consecutive, comparative, non-randomized, interventional case series. Thirty consecutive eyes from 30 patients with subfoveal neovascularization associated with age related macular degeneration were treated by photodynamic therapy, followed by the intravitreal injection of 20mg/0.1ml TA. Changes in intraocular pressure (IOP) and lens were considered as primary outcome indicators. Floaters and appearance of endophthalmitis were also analyzed.

Results 15/28 eyes (53.5%) needed topical drugs to control IOP at month 12 and at 7/25 (28%) were under topical treatment at month 24. Cataracts developed in 8/25 phakic eyes (32%) during the first year and in 4 further eyes during the second year. In summary, 12/22 (54.5%) phakic eyes completing 24 months follow-up developed cataracts. No case developed endophthalmitis.

Conclusion High dose intravitreal TA was associated with cataracts and IOP elevation. The prevalence of high IOP decreased during the second year.

■ 470

Obestatin potentiates the carbachol-elicited contraction of the iris sphincter muscle

ROCHA DE SOUSA A (1, 2), ALVES-FARIA P (2), SARAIVA J (1), FALCAO-REIS F (3), LEITE-MOREIRA AF (1)
 (1) Department of Physiology, Faculty of Medicine, University of Porto, Porto
 (2) Department of Ophthalmology, São João Hospital, Porto
 (3) Department of Ophthalmology, Faculty of Medicine, University of Porto, Porto

Purpose Obestatin is an amidated 23-aa peptide, part of the ghrelin precursor, which was found to bind specifically to GPR 39. Interestingly, these two peptides appear to have opposite effects. While ghrelin stimulates appetite by acting on its receptor in the arcuate nucleus, obestatin suppresses appetite. Obestatin decreases the contractile activity of jejunal muscle strips and antagonized the stimulatory effect of ghrelin. Previously our group had demonstrated that ghrelin relaxes the carbachol precontracted iris sphincter muscle through a GHR31a independent release of prostaglandins. This study investigates the effect of obestatin in the contraction of the iris sphincter muscle.

Methods Rabbit iris sphincter muscles (n=12) were dissected and attached to a force transducer, on a horizontal organ bath containing a modified Krebs-Ringer solution (1.8 mM Ca²⁺; 35°C). The effects of obestatin were evaluated on the resting tension of the muscle in a dose-response curve (10⁻⁹ – 10⁻⁵M; n=6). In a second protocol the effects of obestatin were evaluated in two consecutive contractions elicited by Carbachol (10⁻⁶M; n=6). Only significant results (mean±SE, p<0.05) are given, expressed as % changes from control.

Results Obestatin did not alter the resting tension of the iris sphincter muscle. Carbachol increased active tension from 0.56±0.05 mN to 1.49±0.09 mN. In presence of obestatin active tension increased more 24.8±10.9% in response to carbachol.

Conclusion Obestatin potentiates the Carbachol elicited contraction of the iris sphincter muscle. Similarly to what was described in jejunal muscle strips, obestatin appears to have an opposite of the described to Ghrelin's, in the iris sphincter muscle.

■ 471

In vitro potency and stability of vancomycin eye drops

KARAMPATAKIS V (1), PAPANIKOLAOU T (1), GIANNOUSIS M (2), KILBASANI M (1), GOULAS A (3), MANDRAVELIK (2), ALEXIOU-DANIEL S (2), MIRTSOU V (3)
 (1) Laboratory of Experimental Ophthalmology, Aristotle University, Thessaloniki
 (2) Department of Microbiology, AHEPA Hospital, Aristotle University, Thessaloniki
 (3) Department of Pharmacology, Aristotle University, Thessaloniki

Purpose To study the antimicrobial potency against *Staphylococcus aureus* and stability of vancomycin eye drops in vitro for 4 weeks, under the effect of different storage temperatures and light exposure.

Methods Solution of vancomycin 50mg/ml was prepared using Balanced Salt Solution (BSSTM). Two containers of the solution were exposed to light, while two more were protected from this exposure. One container of each couple was kept at room temperature (240 C), while the other was stored in the refrigerator (40 C). In order to evaluate the antimicrobial potency over a 4-week period, the minimum bactericidal concentration (MBC) against *Staphylococcus aureus* was measured. In order to estimate the stability of the solutions over the same period, we measured pH and UV absorbance. Contamination levels were also examined.

Results The antibacterial potency of vancomycin (1.56µg/ml) remained stable throughout the 4-week period. In addition, the measurement of UV absorbance showed that vancomycin was stable under all storage conditions throughout this period. The pH (ranged from 5.43 to 5.62) seemed to have a slight tendency to increase from acidic to less acidic for all the solutions. All solutions remained sterile throughout the study.

Conclusion Therapeutic failures of treating *Staphylococcus aureus* infections have necessitated the use of fortified antibiotic eye drops. Vancomycin eye drops (50mg/ml solutions prepared by reconstituting with BSS), can be used for 4 weeks under all the examined storage conditions.

■ 472

A New In Vivo Rabbit Model that Simulates Human Dosing to Determine the Distribution of Antibiotics in Ocular Tissues

MICHALID JE (1), ROBERTSON SM (2), BROOKS AC (2), JAMES O (2), OWEN GR (2)
 (1) Alcon Laboratories, Ltd., Hemel Hempstead
 (2) Alcon Research, Ltd., Fort Worth, Tx

Purpose Establish a new method to predict the human ocular penetration and distribution of antibiotics following topical dosing of their commercial formulations using a controlled rabbit model that simulates the human eye.

Methods Anesthetised rabbits were given a single topical dose of commercial antibiotic formulations. A 30 min controlled period that mimicked the human eye with manual blinking (4 blinks/min) and tearing (2 µL/min) followed. Following euthanasia, conjunctival, aqueous humor, iris-ciliary body, cornea, and scleral samples were collected. All antibiotic levels were quantified using HPLC.

Results The United States formulations achieved conjunctival and corneal concentrations (µg/g) as follows: moxifloxacin (VIGAMOX) 6.6±0.3 and 50±5; tobramycin (TOBREX) 3.1±1.4 and 20±5; gentamicin (Generic) < 3 and < 3; levofloxacin (QUIXIN) 1.5±0.3 and 18±2; gatifloxacin (ZYMAR) 0.9±0.1 and 8.6±0.4; and trimethoprim (POLYTRIM) < 0.1 and 2±1. The Japanese formulations achieved the following conjunctival and corneal concentrations (µg/g): levofloxacin (CRAVIT) 2.1±0.8 and 12±2; gatifloxacin (GATIFLO) 2.1±0.4 and 11±2; and ofloxacin (TARIVID) 1.6±0.5 and 6.8±0.8. Maximal aqueous humor levels (AQC max; µg/ml) were moxifloxacin (VIGAMOX) 4.3±0.4; levofloxacin (CRAVIT) 0.49±0.06; gatifloxacin (GATIFLO) 0.35±0.04; and ofloxacin (TARIVID) 0.30±0.4 (mean±SE).

Conclusion Moxifloxacin achieved the highest concentration of antibiotic in the ocular tissues. The moxifloxacin concentration was 3-10 times the level of other fluoroquinolones, at least twice the level of the aminoglycosides, and 25 times the level of the antibiotic trimethoprim in the conjunctiva and cornea.

■ 473

Comparative intraocular penetration of a new ofloxacin formulation versus ofloxacin solution in rabbits

RAMOS P (1, 2), FERNANDES AP (1, 2), BAPTISTA A (2), PROENCA R (1)

(1) Ophthalmology Centre, IBILI, University of Coimbra, Coimbra

(2) Laboratório Edol – Ofidaler Produtos Farmacêuticos S.A., Linda-a-Velha

Purpose To compare the intraocular penetration of a topically applied new 0.3% ofloxacin formulation (Laboratório Edol, Portugal), with a 0.3% ofloxacin solution (Floxedol; Laboratório Edol, Portugal) in healthy New Zealand rabbits.

Methods Rabbits were divided into two groups according to the ofloxacin formulation used: Group A received 0.3% ofloxacin solution and Group B, 0.3% new ofloxacin formulation. Tears, aqueous humor, vitreous and blood samples were collected at different time points after administration (Group B). All samples were assayed for ofloxacin levels by high-performance liquid chromatography.

Results Ofloxacin levels in tears, aqueous humor and vitreous were higher in Group B than in Group A. The difference in ofloxacin levels between Groups was found to be statistically significant ($P<0.05$), while in plasma there was no statistical significant difference.

Conclusion The new ofloxacin formulation was well tolerated after topical administration and ofloxacin levels were higher and remained for a long period of time in tears, aqueous humor and vitreous. These results may encourage the clinical use of this new 0.3% ofloxacin formulation in the treatment of external ocular infections.

■ 474

Expression of fibronectins in response to polylactide (PLA) and poly lactide-co-glycolide (PLGA) polymer implants in the rat eye

OKSÁLA E (1), KAARNIRANTA K (1), RÖNKKÖ S (1), HUHTALA A (2)

UUSITALO H (1)

(1) Depth of Ophthalmology, Kuopio

(2) Depth of Medical Sciences, Tampere

Purpose We sought to examine the tissue reactions of biodegradable polymer implants i.e. polylactide (PLA) and poly lactide-co-glycolide (PLGA) in the rat eye.

Methods Wistar rats were anesthetized with 0.6 mg medetomidine/kg and ketamine 50 mg/kg. After temporal cantotomy the gammairradiated GTR membrane material (BM I), PDLGA 50:50 (BM II, molar ratio of lactic and glycolic acid 50:50) or PDLGA 85:15 (BM III, molar ratio of lactic and glycolic acid 85:15) biomaterial films (diameter 1 mm) from INION Oy (Tampere, Finland) were operated into the subconjunctival space. As a marker of fibrosis, stainings of plasma and cellular fibronectins were analyzed by immunohistochemical methods.

Results GTR membrane material (BM I) rather than glycolic acid containing biomaterials PDLGA 50:50 (BM II) and PDLGA 85:15 (BM III) was observed up to one year following in the rat eye. The higher molar ratio of glycolic acid enhanced degradation of the biomaterials. Interestingly, BM II showed the most beneficial tissue biocompatibility in the rat eye, when related to pFn staining levels, while the BM I caused a clear fibrotic process. Despite of different decay time and influence on expression levels of fibronectins, all BMs shared similar macroscopic short-term inflammation.

Conclusion This study provide new data of biomaterial biocompatibility in rat eye. Our findings of the tissue decay of biomaterials and biomaterial-induced fibrosis may help to develope better biomaterials for eye surgery and to find biomaterial owing optimal drug delivery properties.

■ 475

Assessment of poloxamers as new deswelling macromolecules for corneal organ culture

THURET G (1, 2), ZHAO M (1), MANISSOLLE C (1), DUMOLLARD JM (1, 3), PEOCH M (1, 3), ACQUART S (1, 4), GARRAUD O (4), PRADES JM (1, 5), ROBERT PY (6), GAIN P (1, 2)

(1) EA3063 lab 'cell survival and adhesion', Faculty of Medicine and University Hospital, Saint-Etienne

(2) Ophthalmology dpt, Faculty of Medicine and University Hospital, Saint-Etienne

(3) Pathology dpt, Faculty of Medicine and University Hospital, Saint-Etienne

(4) Eye Bank, Etablissement Français du Sang, Saint-Etienne

(5) Lab of anatomy, Faculty of Medicine and University Hospital, Saint-Etienne

(6) Ophthalmology dpt of Limoges, Limoges

Purpose Poloxamers (P) (BASF, Germany) are synthetic block copolymers of various molecular weights, widely used in pharmaceutical industry. We previously described the use of P188 as deswelling agent during organ culture (IOVS2005;46:826-22). Aim: to assess the other P and select the most suitable agents and their optimal concentrations for corneal deswelling.

Methods The P124, 188, 237, 338 and 407 were dissolved in CorneaMax (Eurobio, France) at 10, 5, 2.5, and 1.25% w/v and osmolarity was measured. Experiment 1: assessment of in vitro cytotoxicity (MTT test) of solutions of 350 mOsmol/L on a human corneal endothelial cell (HCEC) line and on primary cultures of stromal fibroblasts. Exp 2: ex vivo deswelling efficiency and inactivity after 48H storage, assessed by measuring thickness, transparency, and ECD on scientific human corneas (Anatomy Lab). Exp 3: comparative tests versus dextran T500 (Corneajet, Eurobio) on paired corneas.

Results P124 was cytotoxic in vitro (94% reduction in HCEC viability) and ex vivo (total EC loss). P188 had a same deswelling effect as dextran T500, but was less toxic. P237 and 338 were as efficient but as toxic as Dextran T500. P407 seemed less effective in deswelling than Dextran but less toxic.

Conclusion P237, 338 and 407 besides P188 seem interesting for corneal deswelling during organ culture. Experiments on larger series with ultrastructural assessment are ongoing to further assess these 4 candidates.

■ 476

N-Vinyl Amides: A Versatile Family of Monomers For Ophthalmic Biomaterials

HENLEY CE, TIGHE BJ

Biomaterials Research Unit, Aston University, Birmingham

Purpose Existing ophthalmic materials are successful in many respects, but have limitations. This poster describes the structures & characteristics of a novel family of monomers used in synthesis of novel hydrogel materials with enhanced properties for ophthalmic applications.

Methods In order to increase the range of monomers available, one needs to seek enhanced monomer properties in the following areas: monomer reactivity, biocompatibility, solvation & partitioning behaviour. Of the many individual monomers & monomer families examined N-vinyl amides were found to exhibit the most advantageous features. They are a versatile family of protein-like amide group containing monomers that can, for example, be manipulated to create reactive amine groups on polymer chains. Using monomer combinations has provided a synthetic pathway to the creation of materials with desirable enhanced properties.

Results N-vinyl amides are advantageously characterised by good hydrophilicity enabling high EWC values, excellent solvation and partitioning properties, & excellent compatibility with a range of other monomers. N-methyl vinyl acetamide, for example is a key component in the recently launched Comfilcon A contact lens. Comfilcon A is said to be the first intrinsically wettable commercial silicone hydrogel contact lens. We believe that this is enabled by NMVA's reactivity ratio which enables it to be evenly distributed within the polymer matrix avoiding large hydrophobic domains. NMVA reactivity ratios with HEMA lie between NVP $r_1=4.43$ $r_2=0.04$ & NNDMA $r_1=1.56$ $r_2=0.34$ – an isomer of NMVA.

Conclusion This poster demonstrates the great potential of N-vinyl amides in ophthalmic biomaterials development.

■ 477 / 3437

Merkel cell carcinoma of the eyelid with two cases of spontaneous regression

MISSOTTEN GS, DE WOLFF-ROUENDAAL D, DE KEIZER RJW
Ophthalmology, Leiden University, Leiden

Purpose To evaluate the clinical and histopathological characteristics of Merkel cell carcinoma (MCC) of the eyelid and to report cases of spontaneous regression of a MCC after biopsy. The Merkel cells are present in the epidermis and are associated with touch receptors.

Methods Clinical records and histopathological material of patients with eyelid Merkel cell carcinoma were reviewed. The clinical presentation and treatment were evaluated.

Results Three cases of Merkel cell carcinoma of the eyelid were found. Diagnosis was made by pathologic investigation and immunohistochemistry (S100, CK20, EMA, chromogranin). Clinical differential diagnosis must be made with a chalazion, and histopathological differential diagnosis with small cell carcinomas. One of the cases showed complete spontaneous regression after biopsy of the tumour. After local excision, none of the MCC had local recurrence, nor regional or distant metastases. Mean clinical follow-up was 50 months. Non-ocular MCC is known to recur in 66% of cases and to give mortality in almost 33%.

Conclusion Merkel cell carcinoma of the eyelid is a rare malignancy, which is not recognised clinically. It should be excised with a large margin (> 3cm). Due to its high rate of recurrence and lymphatic spread, a tight surveillance of these patients remains necessary. The immunological phenomenon of spontaneous regression points out the importance of the immune system in this disease.

■ 478 / 3438

Vascular features of mushroom-shaped uveal melanomas

AMIRYAN AG (1), SAAKYAN SV (1), LEJLUK VG (2)

(1) *Ocular Oncology and Radiology, Moscow Helmholtz Research Institute of Eye Diseases, Moscow*
 (2) *Clinical Physiology Laboratory, State Research Center – Institute of Biophysics, Moscow*

Purpose To study the vascular features of mushroom-shaped uveal melanomas.

Methods 15 patients with mushroom-shaped uveal melanomas (8 men and 7 women; age varied from 19 to 72 years) were examined using high-frequency (10-13 MHz) duplex ultrasonography with Color Doppler imaging (CDI) and spectral Doppler analysis. The examination was performed using Sequoia-512 (Siemens AG, Germany) with linear array 15L8w. Tumor thickness average was 7.7 ± 2.6 mm (4.3 - 12.4).

Results Studying mushroom-shaped uveal melanomas by CDI, we determined two types of tumor's vascularization. In the eyes with the first type of mushroom-shaped melanomas (8 persons) vascular patterns were detected at the base and in the neck of tumors, whereas there was no abnormal blood flow signals in the head marked. At the patients with the second type of vascularization (7 persons) uniform distribution of Doppler signals in the tumor were visualized. The first type of mushroom-shaped uveal melanomas characterized by significantly more higher peak systolic velocities and lower peripheral resistance in the tumor vessels than the second one.

Conclusion The received Doppler characteristics distinctions of blood flow in the submitted vascular types of mushroom-shaped uveal melanomas could testify the greater tumor activity at the patients with the first type of vascularization. This technique may be valuable as an additional noninvasive tool for the selection of therapy methods the patients with mushroom-shaped uveal melanomas.

■ 479 / 4337

Optico-reconstructive operations in block excision of iridociliar tumours

SAAKYAN SV (1), CHENTSOVA EV (2)
 (1) *Ophthalmology and Radiology, Moscow*
 (2) *Eye Trauma, Moscow*

Purpose The development of iridociliar tumours is often followed by complications which worsen the functional outcomes after the tumour excision. Cataract is one of these complications. The aim was to determine the indications and contraindications to simultaneous optico-reconstructive operations in block excisions.

Methods 571 patients with local iridociliar tumour excision were analyzed. Reconstructive operations were performed in 262 patients. Block excision with the simultaneous cataract extraction was performed in 32 patients (5.6%). During the last two years the lens removal has been followed by intraocular correction in 8 patients.

Results The simultaneous tumour excisions and reconstructive operations have let us preserve the high visual acuity in 65.7% of cases in a mean follow-up period of 10.5 years (from 6 months to 20 years). Iridoplasty has been performed in the tumours of size less than 1/3 of the iris. In all cases the eyes were preserved not only as cosmetic but as functioning organs. The by-layer and through sclerokeratoplasty has been performed in the iridociliar tumours, invading sclera and limb. Intraocular correction was performed in iris with defect of less than 1/3 of its area and in this case the first step was tumour excision, then lens removal, iridoplasty and afterwards intraocular lens was inserted and fixed to the sutured iris.

Conclusion Thus the main contraindication for the block excision done simultaneously with intraocular correction are the excised tumour's volume and area of iris defect. The combined method of tumour excision and simultaneous cataract extraction plus intraocular lens insertion has allowed to acquire high visual acuity in 72% of patients and to preserve eye form and size in all the cases.

■ 480

Uveal lymphoma with extraocular involvement

GARCIA-ALVAREZ C (1, 2), SAORNIL MA (1, 2), MENDEZ MC (2), VALLE ML (3), LOPEZ-LARA F (3), BLANCO G (1, 2)
 (1) *Ophthalmology, Hospital Clínico Universitario, Valladolid*
 (2) *Ocular Pathology Laboratory, IOBA, Valladolid*
 (3) *Radiotherapy, Hospital Clínico Universitario, Valladolid*

Purpose Intraocular lymphomas are a rare pathology presenting as masquerade syndrome and intraocular biopsies used to be necessary for accurate diagnosis. Primary extranodal lymphomas of the uvea are exceedingly rare non Hodgkin B-cell low grade the lymphomas and intra and extraocular affection is a rare condition. We present a case diagnosed by biopsy of the extraocular component.

Methods The patient is a 68 year-old man with decreased visual acuity in left eye presenting with a superior temporal vascularized conjunctival lesion. Left eye funduscopic exhibit an inferior retinal detachment. B-ultrasound scanning showed a diffuse choroidal thickening with a extrascleral component. Orbital CT scan and MR showed a choroidal mass, extending into intraconal fat. Systemic work up was negative for systemic disease. Incisional biopsy of the conjunctival and intraconal mass was performed.

Results Pathology study showed an extensive and dense lymphoid proliferation composed of small cells with MIB-1 lower than 10%. Immunohistochemistry showed marked positivity to B-cells (CD20) and kappa chains, poor for T-cells (CD3) and negative stain for lambda chains. These findings were consistent with low grade non Hodgkin B-cell lymphoma. The patient was treated with low doses external radiotherapy. After 9 months of follow up, intraocular and extraocular mass regressed.

Conclusion Primary uveal extranodal lymphomas are the most uncommon intraocular lymphomas. Diagnosis has been previously performed in enucleated eyes or through intraocular biopsy. In the present case biopsy of extraocular component allow to make the diagnosis, preventing the secondary effects of intraocular surgical procedures. Partially funded by grant "JM Aguilar Bartolome"

■ 481

Bilateral ocular leukemic infiltrate

BOGDANICI C (1), MIRON I (2), DUMITRASS S (2), SARBU L (1), MOTOC I (1)

(1) Dept. of Ophthalmology, Iasi

(2) Dept. of Pediatric Oncology, Iasi

Purpose To report the ocular aspects after telecobaltotherapy of a child with acute lymphoblastic leukemia (ALL) who had bilateral relapse in ocular sanctuary.

Methods We report a thirteen-years old boy who was diagnosed with T ALL. He developed an isolated relapse at the ocular sanctuary after one year of chemotherapy. Clinical parameters: visual acuity, MRI, ophthalmoscopy aspects, intraocular pressure (before and after radiotherapy).

Results The child presented high leukocyte count ($320 \times 10^9/L$), without SNC involvement. The leukemia was placed in the high risk group and the treatment was according to the protocol FRALLE 2000 T. The early response to treatment was good; the bone marrow specimens obtained 14 days after multiagent chemotherapy and at the end of induction phase revealed chemosensitivity and hematological remission. After one year of chemotherapy he developed an isolated ocular relapse involving both eyes; the child had ocular pain and ocular diagnosis was proved after ophthalmologic and MRI evaluation. Ophthalmologic exam showed decreased visual acuity (especially in LE), bilateral leukemic retinopathy and left optic nerve infiltration assessed by MRI. At the moment of the ocular relapse, the morphological CSF and bone marrow analysis showed no evidence of relapse but this possibility could not be excluded due to the lack of detection of molecular target, MRD. He received localized irradiation with improvement of the visual function of the right eye ; the vision of the left eye is still impaired. Ophthalmoscopic aspects were improved for both eyes.

Conclusion The diagnosis and treatment of a leukemic patient require a multidisciplinary approach. The leukemic infiltration of the optic nerve is an emergency and the radiotherapy can improve the visual function.

■ 482

Choroidal biopsy and ultrasound: importance in diagnosis of indeterminate intraocular melanoma. Case report

SANCHEZ CHICHARRO D, SAORNIL MA

Ophthalmology, Valladolid

Purpose Diffuse choroidal melanoma has no specific clinical pattern and sometimes it is difficult to differentiate from other intraocular lesions producing choroidal thickening like subretinal haemorrhage. Choroidal biopsy and ultrasonography are crucial in these cases.

Methods A 77-year-old female was diagnosed of retinal detachment and vitreous haemorrhage after cataract surgery in the right eye, therefore a vitrectomy and scleral buckle was performed not obtaining a successful outcome during the follow up. A year afterwards, a pigmented inferior subretinal lesion was observed measuring 3.45mm height and 12.4mm base under B-ultrasound.

Results The clinical examination together with the ocular ultrasound and the MRI suggested a choroidal melanoma, not ruling out a possible subretinal haemorrhage. A choroidal biopsy was performed leading the diagnosis to choroidal melanoma. Histopathology of the enucleated eye was informed as a diffuse choroidal melanoma with extraocular extension, not detected by the MRI.

Conclusion Intraocular biopsy is a highly useful diagnostic test in the differential diagnosis of selected cases of uveal melanomas. On the other hand, ultrasonography is essential to be performed when media opacities or retinal detachment are present in order to rule out underlying tumoral pathology.

■ 483

Ethmoidal melanoma with orbital invasion

GARCIA-ALVAREZ C (1, 2), BLANCO G (1, 2), SAORNIL MA (1, 2),

MENDEZ MC (2), VALLE ML (3), SANTOS J (4)

(1) Ophthalmology, Hospital Clínico Universitario, Valladolid

(2) Ocular Pathology Laboratory, IOBA, Valladolid

(3) Radiotherapy, Hospital Clínico Universitario, Valladolid

(4) ENT, Hospital Clínica Universitario, Valladolid

Purpose Secondary tumours of the orbit are among the most common orbital malignancies. Orbital invasion from primary neoplasies of the paranasal sinuses is relatively frequent. Primary sinus malignancies showing orbital invasion are usually of epithelial origin. Primary melanomas of the paranasal sinuses are extremely uncommon. The purpose of this paper is to present a case of nasal melanoma with orbital extension.

Methods Interventional case report. Clinical study, CT scan. Light microscopic study and immunohistochemical stains were performed.

Results The patient is a 96-year-old female with progressive exoftalmos and superior and nasal displacements of the left eye. CT scan showed a left ethmoidal mass with frontal sinus, nasal duct, both frontal sinuses and left maxillary sinus involvement. Massive orbital invasion and eye globe tenting and compression was presented. Endonasal biopsy was performed. The histopathology study revealed pigmented epithelioid cells. Immunohistochemistry showed positivity to S-100 and HMB 45 markers, and negativity to CD 45, cytokeratins (AE1AE3) and neurofilamentes. These findings were consistent with a malignant melanoma. Because the age of the patient, extension of the disease, and poor prognosis, external palliative radiotherapy was applied, with significant mass decrease.

Conclusion The most common cause of progressive mass in the orbit is metastasis. The most common etiology of melanoma invasion in the orbit is uveal melanoma. Sinus melanomas are very rare, nevertheless, they very commonly show orbital invasion. Their prognosis is very poor, and usually only palliative treatment is indicated. Partially funded by grant "JM Aguilar Bartolome"

■ 484

Recurrent primary conjunctival melanoma with nasolacrimal extension

DE KEIZER RJW (1, 2), DE WOLFF ROUENDAAL D (1)

(1) Ophthalmology LUMC Ocular Oncology unit, Leiden

(2) Ophthalmology, Antwerp

Purpose Presentation of 6 cases with recurrent conjunctival melanoma with extension to the lacrimal duct and/or intranasal turbinate

Methods Retrospective study in 200 cases of Dutch conjunctival melanoma patients, from which 100 were treated in the LUMC.

Results Six of the 200 patients had a recurrent melanoma and tumour extension to the nasal lacrimal drainage system. All cases showed a long patient and doctors delay in spite of regular frequent control visits with extensive local examination. In 3 patients local radical ENT en bloc resection was performed with postoperative radiotherapy in 2 of them. In 3 patients wide orbital exenteration was performed: in 2 of them the lacrimal region and drainage system was radically excised in the same operation, while in the other case radical excision was done 6.5 years after orbital exenteration without lacrimal sac excision but with radiotherapy performed in another clinic. Peroperative and histological examination of the epithelium adjacent to the tumor failed to show either atypia or melanosis. The melanomas in the nasal cavity are most likely an evolving regional metastasis. Although life expectancy seems short, we observed only one fatal outcome; the longest survival was 17 years.

Conclusion Conjunctival melanoma extension in the lacrimal drainage system either via the tear flow or via direct melanoma growth in the surrounding area is easily overlooked. Inspection and palpation of the lacrimal system and inquiry for nasal bleedings is essential to assess and treat loco regional spread. Promptly treatment might be life saving.

■ 485

Comparison of Diagnostic Imaging Techniques in the evaluation of uveal melanoma

DE FRUTOS-BARAJA JM (1, 2), SAORNIL MA (3, 2), ALMARAZ A (4),
GARCIA-ALVAREZ C (5), BLANCO G (5, 2), LOPEZ-LARA F (6)

(1) Radiotherapy (Medical Physics), Hospital Clínico Universitario, Valladolid

(2) Ocular Oncology, Instituto Universitario de Oftalmobiología Aplicada, Universidad de Valladolid, Valladolid

(3) Ocular Oncology, Hospital Clínico Universitario, Valladolid

(4) Epidemiology and Preventive Medicine, Universidad de Valladolid (Valladolid), Valladolid

(5) Ophthalmology Department, Hospital Clínico Universitario, Valladolid

(6) Radiotherapy and Oncology, Hospital Clínico Universitario, Valladolid

Purpose Measurements of uveal melanomas are crucial for planification and dosimetry when any radiation therapy modality is selected as treatment. The purpose of these paper is to compare the measurements obtained by different diagnostic imaging techniques used in evaluation and treatment of uveal melanomas.

Methods Retrospective study of 50 patients enucleated due to uveal melanoma. The data of the maximum basal diameter and height of the tumor in the macroscopic specimen and the microscopic slide have been used as gold standard. Clinical data (location, shape), obtained by ophthalmoscopy, B-ultrasound, computerized tomography (CT) are considered. The data were collected in a Microsoft Access database and analyzed with the statistical application SPSS and coefficient of correlation of Pearson was applied.

Results Macroscopic vs. microscopic (maximum basal diameter)

General results 0,799 Mushroom Shaped 0,584 Dome shaped 0,584

Anterior 0,809 Posterior 0,793

Macroscopic vs. US and CT (apical eight)

General Mac vs US: 0,866 Mac vs CT: 0,87

Anterior Mac vs US: 0,692 Mac vs CT: 0,95

Posterior Mac vs US: 0,849 Mac vs CT: 0,83

Conclusion Correlation between the diagnostic imaging and histologic measurements are influenced for tumor location and shape: B-ultrasound is the election technique for posterior tumors, and CT scan for anterior melanomas. Partially funded by grant "José M. Aguilar Bartolomé" and FIS 01/1664 (Ministerio de Sanidad y Consumo, España)

■ 486

Malignant transformation of retinocytoma: successful conservative treatment

DE FRANCESCO S (1), CERASE A (2), MENICACCI F (3), FRUSCHELLI M (3), HADJISTILIANOU T (1)

(1) Ophthalmology - Oncology Unit, Siena

(2) Neuroradiology Unit, Siena

(3) Ophthalmology, Siena

Purpose To report two cases of retinocytoma who underwent malignant transformation and successfully treated with chemoreduction, focal therapy and external radiotherapy.

Methods Data on 11 patients with retinocytoma were reviewed for clinical features and malignant transformation.

Results Among 400 consecutive patients who had retinoblastoma, retinocytoma or both, we identified 12 tumors in 11 patients with clinical features compatible with retinocytoma. Two cases underwent malignant transformation into retinoblastoma. Conservative treatment included 6 cycles of chemoreduction with focal therapy and external radiotherapy. Complete regression has been achieved in both cases and the follow-up is respectively of 3 and 2 years.

Conclusion Retinocytoma is a benign retinal tumor with characteristic clinical features. Signs of malignant include: changes in color and size, appearance of a fine vascularization on the surface of the mass and focal vitreous seeding. Close follow-up of retinocytoma is mandatory particularly in patients diagnosed in the first 2 years of life.



2006 | October 4 - 7, 2006
Vilamoura, Portugal

www.ever.be

COURSES

■ 1311

Statistics

KIVELÄ T,DAMATO B

Typical pitfalls, seldom addressed during basic courses in statistics, await the unwary scientist who is starting research in ophthalmology. These include proper ways of analysing data that is collected from both eyes of patients, proper ways of summarising and comparing visual acuities, and recognising and properly analysing time-to event type data in follow-up studies. Moreover, senior scientists who regularly review manuscripts are aware of certain problems in presentation of research data that should be trivial to solve, but which are surprisingly persistent. These comprise recognising the often neglected yet common problem of making multiple comparisons, how to differentiate statistical from clinical significance, how to communicate effect size, and how to cope with statistically non-significant results. This course will clarify the problems mentioned, based on examples from real life and from a practical rather than mathematical point of view. It will provide guidelines for clear presentation of key aspects of the results. The course will specifically address principles that help in reporting statistical results in an informative way.

■ 1321

Acute anterior uveitis and keratouveitis

KESTELYN P. DE SCHRIVER I

Department of Ophthalmology, Ghent University Hospital, Gent

ABSTRACT NOT PROVIDED

■ 1322

Secondary Glaucoma

BRON A M

Dpt of Ophthalmology, University Hospital, Dijon

Purpose Raised intraocular pressure and glaucoma are frequently seen in uveitis, and may represent a serious complication. Inflammatory substances released during uveitis and the treatments used, mainly corticosteroids, probably alter the normal anatomic structure of the anterior chamber and angle, influencing aqueous dynamics. The clinical presentations may vary according to the irido-corneal angle. In angle closure, iris bombe is well recognised due to the acute elevation of IOP. However when the angle remains open, a careful monitoring of IOP and optic nerve head are needed, because the evolution is more insidious. Therefore gonioscopy is the key examination in the diagnosis and management of secondary IOP elevations and glaucomas and allows an appropriate treatment.

■ 1323

Acute non infectious posterior uveitis

COCHEREAU I

Ophthalmology, Angers

Purpose Acute posterior non infectious uveitis may occur mainly in Behcet's disease, sarcoidosis, multifocal choroiditis, sympathetic ophthalmia, Vogt-Koyanagi-Harada syndrome, serpiginous choroidopathy and masquerade syndromes. An immediate antiinflammatory effect is required to limit the lesions induced by the acute inflammation. Only intravenous pulses of methylprednisolone can provide such a rapid effect. The pulses are administered on an outpatient basis, once a day in the morning for three days, and relayed by an oral corticotherapy 1 mg/kg/d. Intravitreal steroids are being evaluated. If anterior inflammation is associated, cycloplegic and local steroids are prescribed to prevent posterior synechiae. Hypotensive drops are administered if needed. If the vitritis is too dense to see the fundus, pulses of steroids may clear the vitreous, and disclose a masquerade syndromes or infection. First-line vitrectomy is not indicated as an emergency anti-inflammatory treatment. If required by the history, additional second-line immunosuppressors and/or immunomodulators can be initiated. But as they need two to three months to achieve their full effect, concomitant corticotherapy must be maintained initially at high dosages. Infliximab, an anti-TNFalpha drug, appears to be a promising immunosuppressant. Interferon alpha is currently used, especially in Behcet's disease. Both drugs and others like mycophenolate mofetil, tacrolimus are currently being tested. Azathioprine, methotrexate, cyclosporine, cyclophosphamide, high-dose immunoglobulin are more conventional treatments. In acute posterior non infectious uveitis, steroids remain the first-line non specific therapy. More specific modulators of the inflammation can be associated for long term treatment.

■ 1324

Ocular toxoplasmosis threatening visual function

KODJIKIAN L

Dept of Ophthalmology, Croix-Rousse Hospital, Lyon

Purpose Ocular toxoplasmosis due to Toxoplasma gondii is presumably the most frequent infectious cause of posterior uveitis throughout the world. Ocular toxoplasmosis is characterized by recurrent episodes of necrotizing retinochoroiditis thought to be caused by both proliferation of live organisms that emerge from tissue cysts within the retina and an associated inflammatory reaction. Toxoplasmic retinochoroiditis can be associated with severe morbidity if disease extends to structures critical for vision, including the macula and optic disk, if there is damage to the eye from inflammation, or if there are complications such as retinal detachment or neovascularization. Clinical diagnosis is helped by ancillary laboratory testing. Witmer-Desmonts coefficient and western blot. The current approach to treatment of severe toxoplasmic retinochoroiditis involves finite courses of anti-Toxoplasma gondii drugs, with or without corticosteroids, in emergency. New strategies for treatment of ocular toxoplasmosis are tested and will be developed during present course.

■ 1325

Viral uveitis and retinopathies

BODAGHIB, LEHOANG P

Ophthalmology, University of Paris VI, Paris

Purpose Rapid diagnosis and treatment of patients with viral uveitis or retinopathies is mandatory in order to prevent severe and sight-threatening complications. Even though oral acyclovir is usually proposed in patients with anterior uveitis, it is frequently insufficient in those with acute retinal necrosis or progressive outer retinal necrosis syndromes. A more aggressive strategy based on major antiviral drugs, such as ganciclovir or foscarnet, or associations of intravenous and intravitreal antivirals is frequently necessary. Corticosteroids should be used carefully and never before stabilization of necrotizing retinitis. Analysis of ocular fluids by PCR is the best method to confirm the control of viral replication before the initiation of any type of anti-inflammatory strategies. 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. Poor visual outcome in patients with necrotizing retinopathies may be associated with an optic disc atrophy. Finally, duration of antiviral therapy after healing of retinal necrosis remains the main issue in patients with severe forms of the disease.

■ 1326

Retinal detachment in patients with uveitis

DE SMET M (1, 2)

(1) Division of Vitreo-retinal and Uveitis, Department of Ophthalmology, ZNA
Middelheim, Antwerp

(2) Department of Ophthalmology, University of Amsterdam, Amsterdam

Purpose Retinal detachment among uveitis patients is an infrequent occurrence - present in 2% of non infectious uveitis patients with less than half having active disease at the time of presentation. Managing these cases requires specific attention to the inflammation when present, as well as the consequences of such inflammation if it develops subsequently in patients. The current present will review present knowledge in the literature.

Methods Literature review and review of own cases operated over a 10 year period. Cases were analysed based on the etiologic diagnosis, characteristics of the detachment, presence of PVR, level of inflammatory activity, immunosuppressive therapy. Outcome measures were visual acuity, anatomic outcome, presence or absence of hypotony.

Results In non inflamed eyes, retinal detachment repair leads to good anatomic results and good recovery of vision. In eyes with ocular inflammation, results are more mitigated with more recurrent detachments due to PVR. In patients with detached maculas, vision recovery after re-attachment is more limited. Better results were achieved with an aggressive approach which included shaving the vitreous base, and peeling the ILM overlying the macula.

Conclusion Repairing detachments in uveitis patients remains a challenge, particularly when the detachment occurs concurrently with intraocular inflammation.

■ 1331

Basics of Electrophysiological testing

HAWLINA M

University Eye Clinic, Ljubljana

ABSTRACT NOT PROVIDED

■ 1332

ERGs in inflammatory disease

HOLDER GE

Moorfields Eye Hospital, London

Purpose The presentation will discuss the place of electrophysiology in the diagnosis and management of patients with inflammatory retinal disease. The objective assessment of retinal, macular and optic nerve function enables the severity of disease to be quantified and can resolve any discrepancy between the nature of symptoms and signs. Electrophysiology can influence management decisions by objectively monitoring disease progression and the efficacy of treatment, and in some circumstances can suggest when to initiate treatment.

■ 1333

ERGs in acquired retinal disease

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 electroretinography in acquired retinal disease.

Methods A case presentation format will be used to illustrate how electroretinography can help in differentiating between several acquired retinal conditions.

Results ISCEV-standard full-field flash electroretinography and pattern electroretinography contribute significantly to making the correct diagnosis in the retinal clinic. Additionally, these tests allow for more accurate predictions regarding the visual prognosis of patients.

Conclusion ISCEV-standard electroretinography is an essential tool in the retinal clinic.

■ 1334

Extended ERG techniques

HOLDER GE

Moorfields Eye Hospital, London, UK

Purpose Since its introduction in 1989, the ISCEV Standard for ERG has been highly successful in encouraging compatibility of basic ERG recordings across laboratories. However, it is only intended as the minimum data set to be obtained in all patients, and there are a number of disorders where confining an ERG investigation to the ISCEV Standard will not only enable an accurate diagnosis, but will, in some instances, probably result in the wrong diagnosis. The presentation will examine the clinical uses of additional ERG techniques such as extended dark adaptation, the use of coloured stimuli, variations in inter-stimulus intervals, ON/OFF and S-cone ERG recording.

■ 1341

Basics in automated perimetry, the glaucoma progression analysis software and case discussions

DAYANIR V

~, Aydin

ABSTRACT NOT PROVIDED

■ 1342

Perimetry in children

MARTIN L

Clinical Neuroscience, Stockholm

Purpose To describe computerized perimetric methods suitable for examining children and present results from normal subjects and pathological cases.

Methods Several new perimetric techniques have been developed during the last decades. The examination procedure has been shortened and a variety of different stimuli and setup configurations are now available. Yet manual perimetry using the Goldmann perimeter is still the most common method when examining children. The experience from using four computerized perimetric techniques, the Humphrey Field Analyzer, High-pass Resolution Perimetry, Frequency Doubling Technology and Rarebit perimetry, in children will be discussed.

Results The optimum method for a particular child is dependent of age, maturity, type of disorder and the examiner's experience. Findings from healthy children and children with glaucoma or neurological disorders, examined with the different techniques, will be presented.

Conclusion In adults, computerized static perimetry has become "gold standard" in most diagnosis. Children can perform computerized perimetry from the age of 7 to 10, depending on method used.

■ 1343

New Developments in perimetry: Frequency Doubling Technology and Blue on Yellow SITA

OZDEN R

~, Danville, CA

ABSTRACT NOT PROVIDED

■ 1344

Interactive case discussion

AYDIN P

~, Ankara

ABSTRACT NOT PROVIDED

■ 1361

Web-Based Resources in Ophthalmology: Resources and Strategies

*SIEVING Pamela
Bethesda*

The Web presents both opportunities and challenges.

This course will help you save time and maximize results when you seek information.

Topics to be covered:

- PubMed: content; search techniques; how to access resources identified when you search
- Other bibliographic databases: Embase, Scopus, Web of Knowledge, LILACS
- Best sources for evidence-based medicine
- Genetics resources
- What is free, and how to get it
- New information resources: Google and Google Scholar; institutional repositories; evaluating Web sites

The course is lecture, demonstration, and hands-on.

If you like, submit questions and problems to the instructor beforehand:

PamSieving@nih.gov



2006 | October 4 - 7, 2006
Vilamoura, Portugal

www.ever.be

ORAL PRESENTATIONS

■ 2111**Genetic and environmental determinants of myopia: investigations in the 1958 British birth cohort**

RAHI JS (1, 2), CUMBERLAND PM (1), BHATTACHARYA SS (3), HAMMOND C (4), WEBSTER AR (3), SHAM P (5), PECKHAM CS (1)
*(1) Centre for Paediatric Epidemiology, Institute of Child Health, UCL, London
 (2) Division of Epidemiology, Institute of Ophthalmology, UCL, London
 (3) Department of Molecular Genetics, Institute of Ophthalmology, UCL, London
 (4) St Thomas' Twin Unit, St Thomas' Hospital, KCL, London
 (5) MRC Social, Genetic & Developmental Psychiatry Research Centre, Institute of Psychiatry, KCL, London*

Purpose To elucidate the biological (genetic and environmental) and social determinants of refractive error.

Methods We are undertaking an integrated epidemiological and genetic programme of work on refractive error in the 1958 British birth cohort. This population initially comprised all 17,000 individuals born in Britain during 1 week in 1958. Surviving members have already been followed (by interview and/or examination) at 7, 11, 16, 23, 33, 41, and most recently at age 45 years when DNA samples were also taken. An extensive range of biomedical and social information has thus been obtained and a highly complex longitudinal dataset established in which information on risk factors and potential confounders or effect modifiers was collected without prior knowledge of the ophthalmic outcomes of interest. Ophthalmic clinical measurements and / or interview data on visual function and ophthalmic disorders were collected at each of the follow-up assessments on all cohort members: these include acuity and refraction. We are currently undertaking, in parallel, lifecourse and genetic epidemiological investigations on a random 25% subsample of cohort members.

Results Key findings in relation to myopia will be reported in this presentation at the special interest symposium.

Conclusion The implications of our findings will be discussed in this presentation at the special interest symposium.

■ 2112**Myopia and worldwide variations with ethnicity**

SAW SM (1, 2, 3)
*(1) Community, Occupational and Family Medicine, National University of Singapore, Singapore
 (2) Singapore Eye Research Institute, Singapore
 (3) Ophthalmology, National University of Singapore, Singapore*

Purpose Myopia, a potentially visually disabling disorder, is especially prevalent in Asian countries. Myopia is generally more common in Asians compared with Whites, African-Americans or Hispanics.

Methods A review of studies conducted in different ethnicities was conducted and the literature summarized.

Results The myopia rates in adults above 60 years are 32.7% in Singapore Chinese, but 17.9% in Whites in the United States, and 12.9% in Australia. Amongst Asians, myopia rates are highest among ethnic Chinese in Hong Kong, Taiwan or Singapore, because of possible genetic predispositions and more intense schooling activities with increased amount of time spent on reading and writing. In Singapore, the prevalence rate of myopia in 15,095 male military conscripts varies by ethnicity: 82% in Chinese, 69% in Indians and 65% in Malays. In the United Kingdom (ALSPAC study), the rates of myopia in 7 and 10 year old children are highest in Chinese and Indians, and lowest in Whites.

Conclusion Ethnic variations may suggest that genes or specific ethnic-related lifestyle habits such as attitudes toward school work, may predispose to myopia.

■ 2113**Imaging the myopic eye**

GILMARTIN B (1), SINGH KD (2), LOGAN NS (1)
*(1) Optometry, Aston University, Birmingham
 (2) CUBRIC/Psychology, Cardiff University, Cardiff*

Purpose To demonstrate novel structural features of human myopia using a new technique that depicts the 3-D shape of the human eye using Magnetic Resonance Imaging (MRI) (Singh et al. IOVS, 2006, 47, 2272-2279)

Methods Seven participants, selected to have a wide range of anisotropic errors were scanned using a Siemens Trio 3-tesla whole-body MRI scanner. A T2-weighted MRI (voxel thickness 0.5x0.5x1.0 mm) is optimised to reveal the fluid-filled chambers of the eye. Automatic segmentation and meshing algorithms generate a 3-D surface model, which can be segmented into a variety of morphological parameters and quantified using linear distances, volumes and surface sphericity. Spatial accuracy and repeatability was assessed by comparing respectively MRI-derived axial lengths with those using partial coherent interferometry (PCI) and 10 separate repeat sessions on a -3D female subject. Colour coded 3-D images facilitated quantitative and qualitative comparison of selected structural features.

Results MRI measures of axial length showed an unbiased mean difference across subjects of +0.41mm compared to PCI (mean \pm sd mm 25.04, \pm 2.04 vs 24.63 \pm 1.97). Repeatability (RE) was consistent with voxel thicknesses for ocular volume (6464 \pm 119.16 mm³), ocular width (13.34 \pm 0.16mm) and axial length (23.78 \pm 0.27mm). Whereas data generally confirm the well documented inter-subject structural variability evident in myopia, of note was increased stretch evident in superior temporal quadrants (equatorial) spanning 10 and 20mm longitudinal distance from the corneal pole.

Conclusion The technique provides a comprehensive and robust representation of the architecture of the globe apposite to contemporary research issues in human myopia. Support: Lord Dowding Fund UK; Advantage West Midlands UK.

■ 2114**Familial influences in refractive development**

GLUGGENHEIM JA (1), SAW SM (2), PONG-WONG R (3), HALEY CS (3)
*(1) Cardiff University, Cardiff
 (2) National University of Singapore & S.E.R.I., Singapore
 (3) Roslin Institute, Edinburgh*

Purpose Under the simplest of quantitative genetics models, refractive error (Rx) can be considered as being determined by an additive genetic component G, and an environmental component E (i.e. ignoring G x E interactions and dominance effects). For such a G + E model, heritability is the proportion of the population variation in refractive error due to additive genetic factors. In multiple regression analysis of the variation in Rx in a cohort of children, the R2 value describes the proportion of variance explained by the data. We sought to test the relationship between heritability, R2, and power to detect an environmental risk-factor for Rx.

Methods We simulated datasets consisting of Rx in families comprising 2 parents and 1-2 children, under a G + E model, across a range of heritabilities. Power to detect a subtle environmental risk-factor was assessed in replicate datasets, when heritability was set at a high level.

Results When heritability is low, R2 is mainly limited by whether the appropriate risk-factors can, and have been, measured and modelled accurately. When heritability is high, R2 cannot exceed 0.5 unless siblings are also considered. Power to detect a subtle environmental risk-factor can be improved by taking account of parental and sibling Rx. However, even for the scenario considered here ($h^2 = 0.85$), this gain will generally be modest. A quantitative genetics mixed model approach (REML analysis) does no better than linear regression.

Conclusion For large studies of the epidemiology of myopia, the inclusion of Rx data on parents and siblings should improve R-squared, but is unlikely to substantially improve power to detect environmental risk-factors. Greater power should be possible with the inclusion of genotyping results for myopia-susceptibility alleles.

■ 2115

The twin eye study

HAMMOND C (1, 2, 3)

- (1) *Twin Research & Genetic Epidemiology Unit, London*
- (2) *West Kent Eye Centre, Bromley*
- (3) *City University, London*

Purpose The Twin Eye Study was set up to establish the genetic epidemiology of refractive error, among other phenotypes. Once heritability is established, twins can be used for gene identification by genome-wide linkage analysis, followed by fine-mapping relevant (and replicated) linkage loci, and by association studies of candidate genes.

Methods 506 pairs of twins, 226 monozygotic and 280 dizygotic, mean age 62 years, underwent autorefraction. Heritability was calculated using maximum likelihood modelling. A subsequent genome-wide linkage analysis of microsatellite markers spaced across the genome in 221 dizygotic twin pairs was performed. A combined linkage and association analysis of the PAX6 candidate gene was performed using tagged SNPs. In addition, replication was attempted with another cohort of twins, and fine mapping of a replicated locus was performed.

Results The heritability of refractive error in this cohort was 85%, and this figure has been replicated by twin studies in Denmark and Australia, among others. A genome-wide scan suggested 5 significant loci where myopia susceptibility genes might occur, the highest on chromosome 11p13. A candidate gene study of the PAX6 gene at this location confirmed linkage but found no association, suggesting variation in regulatory regions of this gene rather than mutations in the gene itself might be associated with myopia. Further work on the PAX6 gene in the Singapore SCORM Study is interesting. Fine association of a replicated locus has been performed and will be presented.

Conclusion To date, no known genes are associated with population-based myopia, but genes play a significant part in the variation of refractive error on a population level. However, there are now several possible loci, and several twin and other genetic studies are refining these areas further.

■ 2121

Antigen Specific CD4+CD25+ Regulatory T-Cells Modulate Intraocular Inflammation in a Model of Experimental Uveoretinitis Induced by a Neoretinal Antigen

BODAGHI B (1), TERADA C (2), DE KOZAK Y (3), FISSON S (2), LEHOANG P (1), KLATZMANN D (2), SALOMON B (2)

(1) *Ophthalmology, University of Paris VI, Paris*

(2) *UMR 7087, CNRS UPMC, Paris*

(3) *INSERM, U598 Cordeliers, Paris*

Purpose We have previously described a murine model of experimental uveoretinitis. Regulatory T cells play a role in this model. In this study, we have compared the therapeutic effects of polyclonal versus antigen specific CD4+CD25+ regulatory T cells after intravenous or intravitreal injection.

Methods In mice expressing hemagglutinin (HA) in the retina after subretinal injection of rAAV, uveitis was induced by intravenous administration of 2 x 10⁶ activated HA-specific T cells. These cells were obtained from purified Thy-1.1 TCR-HA CD25⁺ cells and stimulated by irradiated BALB/c splenocytes and HA peptide for 4 days. At the same time or 4 days later, HA-specific or polyclonal BALB/c CD4+CD25⁺ T cells were injected intravenously or intravitreally. A challenge was performed by intravenous activated HA-specific effector T cells, 21 days after the induction of uveitis. Intraocular inflammation was clinically and histologically studied in all animals.

Results CD4+CD25⁺ T cells controlled uveitis only if they were specific for the target antigen (HA). Compared to intravenous injection, the effect observed after intravitreal injection was obtained with low number of cells. Furthermore, protection against a challenge was achieved only after local administration of HA-specific regulatory T cells.

Conclusion Regulation of experimental uveoretinitis may be obtained by using CD4+CD25⁺ T-cells. Specificity and activation status of these cells should be further analyzed in order to develop new in situ therapeutic strategies.

■ 2122

Is Polymerase Chain Reaction Useful in Uveitis?

PROENCA H (1), FERREIRA C (1), MIRANDA M (1)

CASTANHEIRA-DINIS A (1, 2), MONTEIRO-GRILLO M (1, 2)

(1) *Department of Ophthalmology, University of Lisbon, Lisbon*

(2) *Visual Sciences Research Centre, University of Lisbon, Lisbon*

Purpose To evaluate the diagnostic usefulness of aqueous humour (AH) and vitreous (V) polymerase chain reaction (PCR) in uveitis.

Methods Analysis of 124 intraocular specimens (79 AH and 45 V) from 115 uveitis cases by PCR (2003-2006). Sex, race, age, immunological status and clinical diagnosis (based on characteristic findings, serologic and imaging studies and therapeutic trial) were analysed.

Results 55.7% males, 81.7% Caucasian, mean age 39 years, 24.3% immunosuppressed patients (21.7% with AIDS). PCR was positive in 39.2% of cases in AH and in 57.8% in V. PCR results matched clinical suspicion and provided important diagnostic clue in 70.4% of cases (65.8% in AH and 86.7% in V). PCR was positive in 85.7% of immunosuppressed patients' samples while positive in 65.5% of immunocompetent patients' samples. Main etiologic agents identified by PCR were: *Candida albicans* (28.2% in AH and 25% in V), *Toxoplasma gondii* (15.4% in AH and 21.4% in V), HIV (7.7% in AH and 10.6% in V), fungi other than *Candida albicans* (7.7% in AH and 7.1% in V) and *Streptococcus pneumoniae* (5.1% in AH and 7.1% in V).

Conclusion PCR is a powerful diagnostic tool promptly identifying the causative organism in infectious disorders. PCR may also clarify erroneously considered idiopathic or autoimmune disorders. PCR made a valuable contribution to clarify doubtful diagnosis in 70% of cases. PCR analysis of vitreous samples showed better correlation with the clinical diagnosis than aqueous humour samples. PCR analysis of intraocular fluids seems especially useful in immunosuppressed patients who frequently present atypical findings. PCR, however, needs clinical correlation.

■ 2123

PCR in the diagnosis of *Borrelia burgdorferi* in ocular Lyme disease

SILVA BELTRÁN B, BASTOS A, FERREIRA C, MIRANDA M,

CASTANHEIRA-DINIS A, MONTEIRO-GRILLO M

Ophthalmology, Lisbon

Purpose Lyme disease is a multisystemic disorder caused by *Borrelia burgdorferi* a tick-borne infection. Although ocular manifestations of Lyme disease have long been reported, they remain a rare feature. Ocular borreliosis is still an underdiagnosed disorder, partly because of difficulties in the serodiagnosis of the disease. The authors report three cases of Lyme disease diagnosed by Polymerase Chain Reaction (PCR) in the aqueous humour (AH) and vitreous.

Methods PCR was performed in the AH and vitreous of 3 patients with clinical suspicious of ocular Lyme disease. Each patient had a different ocular manifestation: neuroretinitis, scleritis and vitritis. Active infection due to other agents or granulomatosis disease were excluded. Serum analysis by ELISA and Western blot were performed.

Results One of the three patients suspected for Lyme borreliosis was ELISA negative, while all patients showed a positive Western blot. *B. burgdorferi* DNA was detected by PCR in AH of two patients and in vitreous sample of another one.

Conclusion We believe that PCR can be an effective tool for the diagnosis of ocular Lyme infections. Compared with standard laboratory techniques, it is more accurate and reduces the time required to establish the diagnosis. Further studies with a larger number of clinical samples are necessary to assess the sensitivity and specificity of this method.

■ 2124

Bacterial Identification Using PCR In A Large Series Of Acute Post-Cataract Endophthalmitis

CHIQUET C (1), CREUZOT-GARCHER C (2), ROUBEROL F (3), PALOMBIK (1), DENIS P (3), LAFONTAINE PO (2), BENITO Y (4), CORNUET PL (3), THURET G (5), ROMANET JP (1), VANDENESCH F (4)

(1) *Department of Ophthalmology, Grenoble*

(2) *Department of Ophthalmology, Dijon*

(3) *Department of Ophthalmology, Lyon*

(4) *Department of Microbiology, Lyon*

(5) *Department of Ophthalmology, Saint Etienne*

Purpose To improve identification and speciation of bacteria involved in acute endophthalmitis after cataract surgery, using polymerase chain reaction (PCR) technique

Methods This prospective and multicenter study (2004 - 2005) included 82 patients (mean age 72 years, 1.7-96), with endophthalmitis after cataract surgery. Aqueous humor and/or vitreous samples were collected: 100 microL for standard culture (Brain Heart Infusion) and 150 microL for PCR. In 49 patients (60%) a posterior vitrectomy was performed 1-5 days after the first intravitreal injection of antibiotics and vitreous samples were analysed using the same techniques.

Results Microbiological diagnosis was performed on aqueous humor samples in 46% (PCR 35%, cultures 38%) and on vitreous samples in 65% (PCR 64%, cultures 43%). The analysis of the vitreous from vitrectomy showed that the identification of the bacteria was done in 65%, mainly using PCR. Considering all samples, the infectious agent was identified in 66% of the cases. Bacteria were Gram positive in 96% out of the cases (coagulase negative micrococci 48%, *S. aureus* 9%, *Streptococcus* 26%). A coinfection with two bacteria was reported in 2 cases. The correlation between both techniques was excellent.

Conclusion In acute endophthalmitis after cataract surgery, this large series shows a higher proportion of Gram positive bacteria than previously reported. The association of culture and PCR improves the identification of the causative pathogens in the vitreous from biopsy or vitrectomy. These preliminary results also suggest that PCR performed on vitreous samples allow a higher rate of bacterial identification than on aqueous humor samples.

■ 2125 / 214**Antibiotic Resistances of Bacteria Identified in an Endophthalmitis Prospective Study**

BRON AM (1), PASSEMARD M (1), LAFONTAINE PO (1), PECHINOT A (2), CORNUET PL (3), ROUBEROL F (3), ROMANET JP (4), CREUZOT-GARCHER C (1), CHIQUET C (4)

(1) *Ophthalmology, Dijon*
 (2) *Department of Microbiology, University Hospital, Dijon*
 (3) *Ophthalmology, Lyon*
 (4) *Ophthalmology, Grenoble*

Purpose To assess the antibiotic susceptibility of microorganisms identified in the vitreous and/or the aqueous humor of patients suffering from endophthalmitis.

Methods A multicenter prospective study was conducted from January 2004 to June 2005 to evaluate panbacterial Polymerase Chain Reaction (PCR) versus culture. We determined the antibiotic susceptibility of microorganisms identified by conventional identification (Culture in Brain Heart Infusion). Antibiotic resistance testing was performed using the disk diffusion technique in Mueller-Hinton agar or the VITEK2 system. We divided identified organisms into two groups, wild strain (WS) or with acquired antibiotic resistance (AAR).

Results We included 71 organisms from 64 endophthalmitis; twenty eight different microorganisms were identified. Among them, 84.5% were gram-positive Coccis ((staphylococcus 38/71, (53.5%), streptococcus 14/71, (19.7%). We found 53.5% (38/71) of WS organisms and 46.5% of AAR (33/71) with 7 multiresistant strains. Resistances to fluoroquinolone (FQ) were 13.2%, and vancomycin was still effective against 100% of all tested bacteria.

Conclusion This study about antibiotic resistances of microorganisms isolated in endophthalmitis finds an important part of them (46.5%) with AAR. Sensibilities to FQ are higher than in the literature. Micro-organism diversity is important and despite the predominance of gram-positive Coccis, a combination of intravitreous antibiotics is still recommended as the initial empiric treatment of endophthalmitis.

■ 2126 / 215**S. epidermidis: Friend or Foe in Dry Eye- Its Interaction with Goblet Cells**

GRAHAM JE (1), MOORE JE (2), DARTT D (3), BEST R (2), ALARBI M (2), MCGIMPSEY S (2), DIABOLD Y (4), DOWNES CS (1), MOORE CBT (1), MOORE JE (5)

(1) *UU Coleraine, Coleraine*
 (2) *RVH Belfast, Belfast*
 (3) *Schepens Eye Research Institute, Boston*
 (4) *University of Valladolid, Valladolid*
 (5) *Belfast City Hospital, Belfast*

Purpose Commensal bacteria may contribute to the pathogenesis of ocular conditions including dry eye. *S. epidermidis* is the most commonly isolated ocular surface bacteria. This study investigated the interaction of this bacterium with conjunctival epithelial and goblet cells.

Methods Primary conjunctival goblet cells were cultured as previously described by Shatos et al. *S. epidermidis* was isolated from the conjunctiva of both normal and dry eye subjects and a gentamicin invasion assay was performed with the conjunctival epithelial cell line IOBA-NHC and the primary goblet cells.

Results *S. epidermidis* isolated from the conjunctiva of both normal and dry eye subjects were internalised by conjunctival epithelial cells. In comparison when incubated with primary cultures of human goblet cells, internalisation only occurred with bacteria isolated from dry eye subjects. The genetically modified non-pathogenic *S. epidermidis* strain ATCC 12228 was not internalised by either cell type.

Conclusion Two *S. epidermidis* strains isolated from dry eye subjects, demonstrating the ability to invade primary human goblet cells in culture, would suggest such strains possess virulence mechanisms absent in ATCC 12228 and non dry eye isolates. Previous studies have suggested this invasive capacity of *S. epidermidis* may be linked to the increase in numbers of bacterium at a specific site. *S. epidermidis* is the most frequently isolated bacteria from the ocular surface and as a non-pathogenic commensal, may have the potential to become pathogenic when an increase in quantity is present such as that often observed in ocular conditions such as dry eye. This raises the question of whether invasion as a virulence factor may only be exerted by *S. epidermidis* through a quorum sensing mechanism when the bacterial population reaches a high concentration.

■ 2131

Gene therapy promotes corneal graft survival

BARCIA RN, DANA RM, KAZLAUSKAS A

Schepens Eye Research Institute, Harvard Medical School, Boston

Purpose Corneal endothelial cells (CEC) are essential to keep the cornea clear. Loss of CEC is thought to occur in graft failure, particularly in graft failure due to rejection, an immune reaction that targets endothelial cells. We postulate, that CEC loss during graft failure is due to apoptosis. Furthermore, because CEC in vivo are thought to have little regenerative capability, we hypothesize that preventing apoptosis in the donor corneal endothelium will promote cornea graft survival.

Methods Anti-apoptotic genes (Bcl-xL, Bcl-2, p35 and survivin) were cloned into a retroviral plasmid vector. Retroviruses were used to infect CEC. Apoptosis was induced by etoposide or IFN γ and TNF α , and detected by annexin V and Propidium Iodide staining and flow cytometry analysis. For in vivo studies, we used an orthotopic cornea transplant model. BALB/c mice were used as recipients, and C57BL/6 or BALB/c (syngeneic) corneas were used as donors. For transduction of the endothelium, excised corneas were treated with eGFP, or IZsGreen or IzsGreen-Bcl-xL lentivirus. Apoptosis in the graft's endothelium was detected by TUNEL staining and confocal microscopy.

Results Apoptosis of the graft endothelium occurred in rejecting corneas as early as 2 weeks. We found that Bcl-xL, but not other genes, protects CEC from apoptosis. Lentiviral-delivery of Bcl-xL to the corneal endothelium of donor corneas significantly improved the survival of low risk allografts.

Conclusion Graft failure is accompanied by apoptosis of the endothelium. Bcl-xL protects CEC from apoptosis in vitro and promotes allograft survival.

■ 2132

Long term corneal endothelial cell count after Verisyse IOL implantation associated with Penetrating Keratoplasty in the management of pseudophakic bullous keratopathy

GICQUEL JJ, DIGHIERO PL

Ophthalmology Department / Poitiers University Hospital, Poitiers

Purpose To evaluate the long term endothelium survival of corneal grafts in patients with pseudophakic bullous keratopathy who underwent penetrating keratoplasty (PK), former anterior chamber intra ocular lens (IOL) removal, anterior vitrectomy, angle synecchiolysis and Verysize® (Ophtec BV) IOL implantation either fixated over or under the iris.

Methods In a prospective randomized comparative case series, 36 patients (36 eyes) with PK underwent PK followed by an Artisan® / Verysize® IOL implantation. 17 patients (Group 1: G1) had the IOL implanted over, and 19 (Group 2: G2) had it clipped under the iris in a reversed position. PK results were evaluated both clinically and endothelial cell-count was performed with a Topcon non contact specular microscope.

Results Mean follow-up was 22.76 ± 4.2 (SD)(17-30) in G1 and 21.16 ± 5.36 (SD)(13-30) in G2. After 6 months grafts clarity and cellularity values are not statistically different between the two groups. After 12 months endothelial cell-count values are significantly lower in G1.

Conclusion Retropupillary fixation of the Artisan®/Verysize® IOL when combined with PK is a simple technique, respectful of the anatomy and ensuring better long-term survival of the graft.

■ 2133

Development of an experimental model of lamellar and penetrating keratoplasty

FABIANI L (1), BLANCO-MEZQUITA JT (1), MARTINEZ-GARCIA MC (2), BARCIA A (3), FERRARA P (4), MERAYO-LLOVES JM (1)

(1) Instituto Universitario de Oftalmobiología Aplicada, Valladolid
 (2) Cell Biology, Valladolid
 (3) Animal Facilities, Valladolid
 (4) Advanced Center Of Ophthalmology, Bello Horizonte

Purpose The aim of this work is to describe the surgical technique of both, lamellar and penetrating keratoplasty in hens, and its clinical, biophysical and histological correlation studies.

Methods Lamellar techniques (Air big-bubble, Ferrara's dissection using a nylon suture) and penetrating keratoplasty were performed in eyes of Lohmann classic hens. Clinical follow-up to evaluate complications and haze was carried out and light transmittance measurements were taken. Six months after surgery animals were euthanized and the corneas were fixed in 10% buffered formalin. Morphological changes were evaluated by optic (H-E) and Masson trichrome.

Results Sixty percent of animals had a successful keratoplasty outcome according to clinical observations. The number and grade of complications (abscess, loss of corneal buttons, neovascularization and opacities) and the grade of clinical haze were higher in both air big-bubble and Ferrara's dissection than in penetrating technique. Higher values of transmittance were observed in penetrating keratoplasty than both air big-bubble and Ferrara's dissection lamellar keratoplasty. An increase of keratocytes and tissue damage in the interface of lamellar keratoplasty was observed. There were not statistically significant differences in these parameters between air big-bubble and Ferrara's dissection corneas.

Conclusion An experimental animal model of lamellar and penetrating keratoplasty was successfully developed in adult hens. This model could be useful in the study of wound healing and pharmacological modulation after keratoplasty surgery in an animal with Bowman's layer.

■ 2134

Compositional dynamics of phospholipids in the tear film

CAMPBELL D (1), GRIFFITHS G (2), TIGHE BJ (1)

(1) Biomaterials Research Unit, Aston University, Birmingham

(2) CEAC Aston University, Birmingham

Purpose Lipids of the meibomian gland secretions (MGS) have been well characterised but lipids in the tear film (TF) have received less consideration. Phospholipids (PL) of the TF are important as they are thought to act as an interface between the aqueous and lipid layers of the TF providing lubrication due to their low interfacial tension properties. This work addresses the dynamic compositional nature of phospholipids in the TF

Methods Liquid Chromatography Mass Spectrometry (LCMS), Gas Chromatography Mass Spectrometry (GCMS), Thin Layer Chromatography (TLC) were used to identify lipids in the TF

Results LCMS, GCMS and TLC show that PL are present in the tear film at concentrations lower than previously thought. Dittmers solution indicate PL were present at less than 1 μ g. TLC indicate that lysophospholipid (LPC) may be present and that diacylglycerides (DAGs) are present in the TF

Conclusion Tear film PL are reduced in comparison to the MGS PL. DAGs present in the TF may arise through rapid processing of MGS PL by the putative activity of phospholipase C. If LPC is present in the TF then this would suggest the activity of phospholipase A2. We are currently investigating the role of phospholipases in these processes

■ 2135

Corneal endothelial status twenty years after penetrating keratoplasty

BOIRNE WM (1), PATEL S (1), HODGE DO (2)

(1) Mayo Clinic Ophthalmology, Rochester, Minnesota

(2) Mayo Clinic Health Sciences Research, Rochester, Minnesota

Purpose To report the status of the central corneal endothelium in a cohort of patients observed for 20 years after penetrating keratoplasty.

Methods From a prospective cohort study of 500 consecutive penetrating keratoplasties performed by one surgeon between 1976 and 1986, re-grafted eyes and fellow eyes of bilateral cases were excluded, leaving 388 grafts of 388 patients available for analysis. The endothelium was photographed by a specular microscope at intervals after surgery through 20 years. Endothelial cell density (ECD) was measured, and the percentage of cell loss from preoperative was calculated. ECD was fitted to a biexponential model to combine the rapid rate of early cell loss and the slower rate of chronic cell loss.

Results Of 200 patients who were presumed to be alive and without graft failure at 20 years after surgery, 38 patients (19%) were examined. ECD at 20 years was 801 ± 245 cells/mm² (mean \pm SD, n=38), and did not differ from ECD at 15 years (872 ± 348 cells/mm²; paired test, $P=0.84$, n=34, minimum detectable difference = 93 cells/mm², $\alpha=0.05$, $\beta=0.20$). Cell loss from preoperative was $74 \pm 10\%$ (n=38), and the annual rate of cell loss between 15 and 20 years was $0.5 \pm 4.5\%$ (n=34). In the biexponential model of endothelial cell loss, the half-life of the fast component was 9.0 months, and the half-life of the slow component was 285.2 months. Eighty-four grafts were known to have failed by 20 years after surgery, and 26 (31%) were caused by late endothelial failure.

Conclusion The rapid endothelial cell loss in the first decade after penetrating keratoplasty plateaus in the second decade, when endothelial cell loss is similar to normal corneas. Late endothelial failure was the leading cause of graft failure over 20 years.

■ 2136 / 330

Expression of CTLA4-Ig and vIL-10 by gene-engineered dendritic cells leads to prolongation of corneal allograft survival

SCHLICKEISER S (1, 2), RITTER T (3), LI XQ (2, 4), APPELT C (1), VOLK HD (1),

PLEYER U (2), SAWITZKIB (1)

(1) Institute of Medical Immunology, Charité Universitätsmedizin Berlin, Berlin

(2) Dept. of Ophthalmology, Charité Universitätsmedizin Berlin, Berlin

(3) Regenerative Medicine Institute, NUI Galway, Galway

(4) Dept. of Ophthalmology, Huazhong University of Science and Technology, Wuhan

Purpose Systemic adenoviral CTLA4-Ig or vIL-10 gene therapy has been previously shown to be successful in prevention of allograft rejection in experimental keratoplasty. To achieve long-term survival without systemic administration of adenoviral particles this study focuses on the application of ex vivo gene-modified dendritic cells (DCs).

Methods DCs were generated from murine bone marrow. Cells were transduced with an adenovirus encoding vIL-10, CTLA4-Ig or EGFP as a control. Allo-stimulatory capacity of DCs was determined by analyzing the proliferation of co-cultured allogeneic T cells. 7 days prior to experimental keratoplasty 2x10⁶ transduced BALB/c donor DCs were injected i.v. into C57BL/6 mice receiving corneal allografts. A rejection score was daily graded by cornea clarity and oedema. Intra-graft cytokine mRNA expression and presence of Foxp3+ CD25+ CD4+ regulatory T cells in lymphoid organs were analyzed by RT-PCR and flow cytometry.

Results Transduction of DCs with AdCTLA4-Ig prior to LPS stimulation dramatically diminished their allo-stimulatory capacity. Compared to controls, AdvIL-10 transduced DCs showed a significant decrease in T cell activation. Adoptive transfer of CTLA4-Ig transduced DCs resulted in a significant prolongation of graft survival, vIL-10 expressing DCs strongly reduced the rejection rate. This could be confirmed by higher levels of Foxp3+ CD25+ CD4+ regulatory T cells in lymph nodes and spleen of animals receiving CTLA4-Ig or vIL-10 transduced DCs.

Conclusion Adoptive transfer of gene-engineered tolerogenic dendritic cells is a promising approach for the prevention of corneal allograft rejection. (Supported in part by DFG PI 150/14-1/2)

■ 2137 / 331

Prolongation of corneal allograft survival by topical application of Everolimus

PLEYER U (1), BUCH G (2), OTASEVIC L (1), SCHLICKEISER S (3)

BERTELMANN E (1), LI XQ (4)

(1) Department of Ophthalmology, Charité - Universitätsmedizin Berlin, Berlin

(2) Institute of Pharmacy, Charité – Universitätsmedizin Berlin, Berlin

(3) Institute of Medical Immunology, Charité – Universitätsmedizin Berlin, Berlin

(4) Huazhong University of Science and Technology, Wuhan

Purpose Everolimus is a novel proliferation signal inhibitor with potent immunosuppressant effects that holds promise to prevent acute allograft rejection. Commonly, topical application of immune modulatory agents is preferred to its systemic use to reduce side effects of the drug. Here we evaluated the efficacy of topical administration of Everolimus on experimental corneal grafts.

Methods Female Lewis rats received 3.5 mm grafts of DA MHC I/II incompatible donors and were randomly assigned to receive either 0.05% Everolimus in a microemulsion formulation (1), 0.025% Everolimus in a microemulsion formulation (2) or the drug vehicle (3) as control. All treatments were started at the day of surgery and applied five times daily for 35 days. Grafts were graded every day and a rejection score was generated based on cornea clarity and oedema.

Results Local administration of 0.05% or 0.025% Everolimus was effective in prolonging the mean survival time of corneal grafts (MST 21.4 ± 7.0 days, 16.4 ± 2.3 days) as compared to the vehicle control group (13.3 ± 1.7 days; $p=0.006$ and $p=0.015$).

Conclusion These data indicate that topically applied Everolimus significantly prolonged the corneal allograft survival in an experimental keratoplasty model. (Supported in part by DFG PI 150/14-2)

■ 2138 / 332

Comparison of the Visual Acuity by Time Index with conventional Survival Curves for the evaluation of osteo-odontokeratoprostheses functional results

MICHAEL R (1), TEMPRAÑO J (2), CHAROENROOK V (2), DE LA PAZ M (2),

HITZL W (3), GRABNER G (3), BARRAQUER RI (2, 1)

(1) Institut Universitari Barraquer, Barcelona

(2) Centro de Oftalmología Barraquer, Barcelona

(3) Universitätsklinik für Augenheilkunde und Optometrie, Paracelsus Medizinische Privatuniversität, Salzburg

Purpose To analyse the functional results of osteo-odontokeratoprostheses comparing conventional survival analysis with the Visual Acuity by Time Index.

Methods We reviewed 180 charts of patients that underwent osteo-odontokeratoprostheses (OOKP) at the Centro de Oftalmología Barraquer. Mean follow-up time was 8.2 years (range 1 months to 30 years). Kaplan-Meier survival curves with 95% confidence interval (CI) were calculated for functional success defined as BCVA >= 0.05. Visual Acuity by Time (VAT) - Index also with 95% CI was calculated based on the Monte Carlo method. Only one operated eye per patient was included in the analysis.

Results Based on Kaplan-Meier, 5 year functional survival was 53% (CI 45 - 62%). Mean survival time was 10.5 years (CI 8.4 - 12.7 years) and median survival time (loss of 50% of cases) was 5.4 years (CI 3.6 - 7.2 years). According to the VAT-Index calculations, maximum VA can be expected at 1.2 years after surgery with a mean VA of 0.35 (CI 0.29 - 0.41). At 5 years after OOKP, mean VA of all cases was found to be 0.26 (CI 0.20 - 0.34).

Conclusion Survival analysis estimates the time point when VA drops below a predefined value (terminal event) without analysing the time course of VA. The VAT-Index can estimate the VA at a certain time points as well as average VA within arbitrary time intervals after surgery.

= 2139 / 333

**Swelling and deswelling of human corneas during eye banking:
present and future**

THURET G, ZHAO M, GAIN P

Ophthalmology department, EA 3063 'Cell survival and adhesion in cancers and grafts',
Faculty of medicine, Saint-Etienne

Purpose Stromal swelling is a mandatory side effect of human corneal organ culture before graft. After few days of storage, it leads to the doubling of corneal thickness, stromal clouding and dramatic Descemet folding. Causes for this stromal hyperhydration are multiple, mainly the partial loss of functional epithelial and endothelial barrier. The consequences of such a swelling, at a cellular level are not yet fully understood but the triggering of endothelial apoptosis, for instance is very likely, be it direct or indirect by the disturbance of cell adhesion to the Descemet in the folds. To reverse the swelling, corneas have to be immersed in a storage medium supplemented with a high molecular weight molecule. During this step called 'deswelling' the osmotic properties of the macromolecule contribute to extract the water excess accumulated within the stroma. To date, the only macromolecule validated for a routine clinical use is the T500 Dextran, a glucose biopolymer. This corneal deswelling immediately before graft facilitates the suture, dramatically increases the transparency and accelerates the post operative visual recovery. Nevertheless, during this step a very important endothelial cell loss is observed, varying between 7 to 15 % depending on to the deswelling duration. Efforts to replace the T-Dextran have been made during the past few years, using either hydroxyethyl starch (polysaccharide) or poloxamer family (non-ionic branch copolymers of poly(ethylene oxide)/propylene oxide/ethylene oxide) with the hope to reduce the endothelial cell toxicity during the final dwelling step. Poloxamer 188 is currently evaluated in a randomized clinical trial. Another strategy consisting in using hydroxyethyl starch all along the organ culture to avoid stromal swelling from the beginning is also under evaluation.

■ 2141

Lens Crystallin glycation and ascorbylation in diabetes and aging

MONNIER V (1), FAN X (2), RENEKER L (3), JARVIS S (4), OBRENOVICH M (2)

CHENG R (3), ORTWERTH B (3)

(1) *Pathology & Biochemistry, Cleveland*(2) *Pathology, Cleveland*(3) *Ophthalmology, Columbia*(4) *Biosciences, Canterbury*

Purpose Human lens crystallins become progressively pigmented and crosslinked with age, in part due to glycation reactions by reactive carbonyl compounds. We hypothesized this process might be in part mediated by ascorbic acid oxidation products. However, unequivocal demonstration of this concept has not been possible due to the similarity of advanced glycation products (AGEs) from reducing sugars and oxidized vitamin C compounds.

Methods We created a humanized transgenic mouse lens expressing high levels of the human sodium dependent vitamin C transporter 2 (SVCT2) under control of the mouse alpha-crystallin promoter-chick lens delta crystallin enhancer.

Results TG mice revealed sustained lenticular expression of SVCT2, with a 10-20 fold elevation of ascorbic acid (ASA)(1-3 mM) and dehydroascorbic acid (DHA)(0.05-0.3 mM) levels compared to WT. At 6, 9, 12mos, the ASA-derived glycation products pentosidine, vespertlysin A, CML and K2P crosslinks were several fold highly increased compared to wild-type ($p<0.0001$), and significantly increased with age ($p<0.001$), while the glucose-derived Amadori product was normal. The lens protein fluorescence at ex/em 370/440 and 335/385 were also significantly elevated in TG mice with age compared to WT mice. At 12 months old, TG mouse lens showed yellow discoloration that resembles the aging human lens.

Conclusion Longitudinal studies are being carried out to evaluate the long-term impact of accelerated ascorbylation on the crystallin structure and the effect of pharmacological intervention of the process. This study provides the first and unequivocal evidence for the participation of vitamin C degradation products in the chemical aging process of the lens.

■ 2142

Glycation and Cataract

HARDING J

Nuffield Lab. of Ophthalmology, Oxford

Purpose Lens protein glycation followed by the formation of advanced glycation end products and high molecular weight aggregates plays a significant role in the formation of cataracts. The targets of such glycation could be the enzymatic as well as the non-enzymatic proteins such as membrane proteins, leading to a loss of their native conformation accompanied by loss of function. Cataract is hence a conformational disease, like Alzheimer's, and post-translational modifications (PTM) of lens proteins are the most likely cause of the unfolding. However, these proteins can be protected against the deleterious effects of PTM by alpha-crystallin, which acts as a molecular chaperone.

■ 2143

Antioxidants in cataract prevention

HEGDE K, VARMA S

Ophthalmology, Baltimore

Purpose Monovalent oxygen reduction, generating various other reactive species and consequent oxidative stress induces cataractogenesis. Recently, pentavalent oxidation of guanidino-nitrogen generating •NO, another free radical, is also enhanced in diabetes with production of Peroxynitrite (PN). Studies on the mechanism of detoxification of this potent oxidant are limited. We hypothesize that this could be accomplished by homolytic fission of pyruvate (PY) by PN, generating acetate, CO₂ and HONO. HONO is detoxified by endogenous amino compounds by van Slyke reactions.

Methods The decomposition of PN by PY was shown by incubating 1 μmole SIN-1 (morpholinosydnonimine) as a source of PN with 1-14C PY and measuring the formation of 14CO₂. The physiological significance of the finding was assessed by the effectiveness of PY (10mM) in protecting SIN-1/SNAP (1mM) induced damage to the tissue metabolism, reflected by ATP lowering, and by damage to the active 86Rb⁺ transport activity of the mouse lens in culture.

Results Incubation of SIN-1 with 1-14C pyruvate resulted in significant production of 14CO₂ following a first order kinetics at lower concentrations of PY, tending to zero order at higher levels. Physiologically, SIN-1 & SNAP were highly damaging, apparent by the decrease in the ATP levels as well as the activity of Na⁺-K⁺ pump, measured by 86Rb⁺ transport. Both were reversed substantially by pyruvate.

Conclusion 14CO₂ production from pyruvate in presence of PN strongly supports our stated hypothesis. Since the products are innocuous, PY was considered to be biologically useful in detoxifying PN. The physiological significance of the findings was proven further by the PY effectiveness in protecting the tissue against damage to its transport and metabolic activities due to PN induced oxidative stress.

■ 2144

Evidence for protection against oxidative stress in the lens

SÖDERBERG PG (1, 2, 3), DONG X (1), AYALA M (4, 1), MODY VC (1)

LÖFGREN S (1), MEYER L (5, 1), KAKARM (1), WANG J (1), ZHANG F (3, 1)

(1) *St. Eriks Eye Hospital, Karolinska Institutet, Stockholm*(2) *Dept. of Biomedical Engineering, University of Miami, Miami*(3) *Dept. of Ophthalmology, University of Dalian, Dalian*(4) *Dept. of Ophthalmology, Örebro university hospital, Örebro*(5) *Dept. Ophthalmology, University of Bonn, Bonn*

Purpose To review evidence for protection against oxidative stress in the lens induced by UVR

Methods Albino Sprague Dawley rats were exposed to UVR at wavelengths around 300 nm. Damage was measured as intensity of forward light scattering or Maximum Tolerable Dose (MTD). The content of the antioxidant alpha tocopherol and glutathione in the lens was measured with HPLC.

Results UVR exposure is believed to damage the lens through oxidation either directly or through photosensitization. There is evidence for intrinsic post damage protection against UVR induced oxidative stress in the crystalline lens. It was demonstrated that subcapsular osmotic disturbances induced by close to threshold dose UVR may resolve within weeks after exposure. It was further demonstrated that on an average approximately 18 % of the damage induced by a close to threshold dose is repairable while the remaining 82 % is not. The repair was shown to decrease exponentially declining with time, with a time constant (1/e) of 8 days. There is also evidence for a potential for extrinsic pre-damage protection against UVR induced oxidative stress in the crystalline lens. It was shown *in vivo* in rats that pre-treatment with alpha tocopherol increases the lens content of glutathione. Further, the pre-treatment is associated with less light scattering in the lens after exposure to UVR compared, with no pre-treatment. Pre-treatment with alpha tocopherol also was shown to reverse the previously demonstrated increased sensitivity in the lens after 15 min. exposures to UVR.

Conclusion All of the above data strongly support that there is both an intrinsic and a potential for extrinsic protection of the lens against oxidative stress.

■ 2151

Fluidmechanical analysis of microstructural changes in retinal arteries in glaucoma

KOTLIAR KE (1), SCHILLING R (2), VILSER W (3), LANZL I (1)

(1) Department of 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 By image analysis with the Retinal Vessel Analyser (RVA) it is possible to perceive vessels in their dynamic state online non-invasively along a given vessel segment. Previously we demonstrated changes in longitudinal vessel profiles of retinal arterial segments in glaucoma patients and age-matched healthy persons. High-frequency waviness of retinal arterial walls (HFW) and hence the roughness along vessel longitudinal sections increases significantly in glaucoma patients during vascular dilation. Whether these microirregularities might influence hydraulic parameters of a vessel was investigated in this study using the model of non-Newtonian blood flow (blood viscosity depends on its velocity, which reflects the real situation in small vessels) in a retinal vessel segment.

Methods A C++ program was written in order to create 3-D geometry of a retinal arterial segment and corresponding computational grid. This allowed the variation of HFW parameters. Blood rheology was modelled according to Quemada. Finite Volume Modelling procedure was applied for further calculations with computational fluid dynamics code NS3DV43C. Hydraulic resistance along a modelled vessel segment was calculated depending on HFW and other parameters of the waviness.

Results Hydraulic resistance along the modeled vessel segment increased with increasing HFW.

Conclusion Increasing HFW during arterial dilation in glaucoma patients worsens hydraulic conductivity of retinal arteries. The microirregularities in longitudinal profiles of retinal arterial walls found in our clinical studies and analysed here might be an indication for vascular endothelial alterations in glaucoma, leading to impaired perfusion and regulation.

■ 2152

Biomechanical estimation of the optic nerve head parameters, measured with HRT-II in myopia and in its combination with glaucoma

RYABTSEVA AA (1), CHOMYAKOVA EN (1), SERGUSHEV SG (1),

SVETLOVA OV (2)

(1) Department of Ophthalmology, Moscow Regional Medical R&D Institute, Moscow

(2) Department of Ophthalmology St.-Petersburg Medical Academy of Postgraduate Education, St.Petersburg

Purpose Pressure in vitreous chamber (VC) is completely determined through the clinical refraction of a patient and the tonus of his ciliary muscle (CM). In emmetropia at near sight the pressure in VC is minimal, while it is maximal at the far sight. If emmetropic subject is undercorrected his CM is relaxed almost all waking hours. Hence the VC pressure in such eyes is maximal for long. In our previous studies we showed that the excavation of the optic nerve head (ONH) increases if CM is relaxed (Ryabtseva et al., 2005). Collapsing of retinal fibers of ONH at relaxing CM might worsen their state and increase the development of open angle glaucoma (POAG). The aim of the study was to estimate in-vivo the parameters of collapsing of retinal fibers when changing CM tonus with pilocarpine and cyclomed.

Methods Three groups of patients were investigated: hypermetropic subjects (IIa stage), subjects with high myopia and myopic persons with normal pressure glaucoma. Using Heidelberg Retinal Tomograph (HRT) parameters of retinal nerve fibers of ONH were estimated in all patients by application of pilocarpine and cyclomed. Measured tonometric IOP was from 14 to 21 mmHg in all examined persons.

Results Statistically significant changes of mean thickness and cross-sectional area of retinal nerve fibers was found in all investigated groups.

Conclusion Our findings confirm possible influence of eye refraction to the rate of POAG development

■ 2153

Clinical estimation of scleral rigidity for biomechanical modeling of hypotensive non-penetrating sclerotomy

ZASEEVA MV (1), SVETLOVA OV (1), KRASNITSKAYA S (2), KOTLIAR KE (3), KOSHITZ IN (4)

(1) Department of Ophthalmology, Medical Academy of Postgraduate Education, St.Petersburg

(2) Department of Mechanics and Control Processes, Polytechnic University, St.Petersburg

(3) Department of Ophthalmology, Munich University of Technology, Munich

(4) Petercom / MS Consulting Group Cl. corp, St.Petersburg

Purpose Increase of scleral rigidity represents an original reason for natural age-dependent IOP increase in the eye. Our clinical experience shows, that the coefficient of scleral rigidity (K_r) changes from $K_r Y = 2$ MPa (in the young eye) to $K_r A = 6$ MPa (in the aged eye) (Svetlova et al., 2002). The actual IOP level (P_{OC}) correlates well with the actual scleral rigidity level ($K_r C$). Targeted decrease of $K_r C$ in a glaucomatous eye up to individual physiological level of the young eye ($K_r Y$) might lower P_{OC} and retard the development of primary open angle glaucoma (POAG). The aim of presented study was to estimate the $K_r C$ value and the targeted IOP $P_{targ} = P_{OY}$ and to calculate individual anatomical and morphological parameters of the novel hypotensive surgery named non-penetrating sclerotomy.

Methods 130 eyes of POAG patients of different age were investigated. $K_r C$ and P_{OC} were calculated using applanation tonometry by Maklakov with weights of 5g (Pt5) and 10g (Pt10) and classical relationships by Römer (Römer, 1918): $K_r C = (Pt10 - Pt5) / (\Delta V10 - \Delta V5)$ and $P_{OC} = Pt10 - (K_r C \cdot \Delta V10)$. $\Delta V5$, $\Delta V10$ represent changes in intraocular volume during tonometry. Assuming $P_{OY} / P_{OC} \approx K_r Y / K_r C$ the lower value of $P_{targ} = P_{OY} = 2 P_{OC} / K_r C$ was calculated. Based on obtained data numerical Finite Element Model of non-penetrating sclerotomy was developed with commercial code ANSYS.

Results Optimal form, amount and geometry of scleral cuts were estimated, which are needed to provide IOP decrease up to P_{targ} .

Conclusion According to our calculations, the non-penetrating sclerotomy might lower IOP in the glaucomatous eye and retard the development of POAG

■ 2154

On the mathematical modeling of applanation method for measuring intraocular pressure

BAUER SM (1), LYLIBIMOV GA (2)

(1) St. Petersburg State University, St. Petersburg

(2) Moscow State University, Moscow

Purpose Applanation tonometry estimates intraocular pressure (IOP) by quantifying the diameter of the circular contact area of the cornea and flat tonometer of defined load (Maklakov tonometer) or by estimating the force needed to generate a defined amount of deformation of the cornea (Goldmann tonometer). The first simplest models of the applanation method for measurement of the IOP were based on approach, in which an eyeball is modeled as a thin-walled spherical liquid-filled shell. It was assumed that the biomechanical properties are the same for all patients and in the clinic special tables are used to estimate IOP. The calculating of such tables is based on the empirical values of the IOP. But the geometrical parameters of eyes essentially vary for different people and change with age and especially after refractive surgery. So the corneal responses are not fully understood and predictable. And it is interesting to elucidate how corneal and scleral properties may play a role in determining IOP.

Methods : In the model the shell of eye is considered as two segments with different mechanical properties. The two segments shell is filled with uncompressible liquid under the pressure. The deformation of the shell part which models the cornea is significant and to analyze this deformation nonlinear theory of shell is used. The cornea and sclera are considered as transversal isotropic shells. The effect of the physical properties of the shells on the results of the modeling is investigated.

Results The model shows that tonometry readings do not always reflect true IOP values.

Conclusion The real value of IOP could essential depend on the corneal thickness, on the geometrical parameters of sclera and the elastic properties of cornea and sclera.

■ 2155

Modeling the human cornea – a stromal tissue constitutive model based on measured collagen architecture

CHERNYAK DA (1), VAN DER HEIDE DT (2), PINSKY PM (2)

(1) AMO, Inc., Santa Clara

(2) Biomechanical Engineering, Stanford University, Palo Alto

Purpose Disturbances of the stromal microstructure, occurring in refractive surgical procedures such as LASIK, may create unexpected and undesired changes to the vision quality of the eye. With the advent of wavefront-guided LASIK, enabling highly precise measurement and treatment of the defects of the eye's entire visual system, it has become crucial that surgical planning include knowledge of the cornea's mechanical response to the procedure.

Methods The mechanical properties of the cornea derive from the specialized architectural arrangement of its collagen fibrils. The preferred orientation of collagen fibrils throughout the cornea, limbus, and adjacent sclera has recently been mapped using synchrotron X-ray scattering and shows the collagen to have a striking degree of anisotropy in its arrangement. A mathematical description of the data has been derived providing a probability distribution for collagen fibril orientation. It accounts for the strain energy of the oriented collagen, the aqueous extra-fibrillar matrix and the cross-linking energy of the proteoglycans.

Results The elastic constants were calibrated using clinical data for radial keratotomy. The cornea is a prestressed by the action of the IOP. A finite element modeling approach has been developed to determine the prestress and allow true modeling of the surgical process whereby stress is redistributed by surgical cutting of stressed tissue. Results are expressed in Zernike coefficients, and provide measures of optical power and aberrations in the optical zone.

Conclusion The model has been employed to simulate: tunnel incisions in the sclera made for cataract extraction, microkeratome cutting of LASIK flaps, and myopic LASIK procedures.

■ 2156

Mechanical properties of certain eye tissues

IOMDINA EN

Helmholtz Research Institute of Eye Diseases, Dept. of Pathology of refraction and binocular vision, Moscow

Purpose Although biomechanical properties of eye tissues may play a crucial role in varying eye conditions, they are still far from being well studied. Purpose: to study and compare biomechanical parameters of human connective-tissue eye structures (sclera, cornea, lid skin), muscles (upper lid elevator) and conjunctiva.

Methods A testing machine, Minimat 2000, was used to study stress-strain parameters of tissue samples of 16 eyes of humans aged 5-84. Uniform-sized samples of sclera and cornea were cut from different areas of the outer shell of the eyes enucleated after serious eye traumas; other samples were obtained during plastic surgery for ptosis.

Results The elasticity modulus (E) of healthy isolated sclera varies from 2.5 to 26 MPa depending on the patients' age and localization of scleral samples: in the equatorial area it equals 21.6 ± 6.0 MPa and in the posterior pole it falls to 8.4 ± 4.9 MPa. The mean rupture strain of the sclera ($35.0 \pm 9.1\%$) is significantly lower than that of the cornea ($67.3 \pm 10.2\%$). The parameters of upper lid skin are also age-dependent: tensile strength (maximum stress) changes from 10.1 ± 1.2 MPa at 5 years to 12.2 ± 1.7 MPa at 30 and to 4.95 ± 0.9 MPa at 84 years. Age dynamics of E values are similar: 36.1 ± 4.2 MPa, 57.5 ± 6.0 MPa and 15.5 ± 2.3 MPa, resp. Biomechanical parameters of the upper lid elevator are closely connected with the stage of ptosis: at a given age, the maximum stress and elasticity modulus are approximately twice as low in severe ptosis as in the initial stages, which testifies to the significant role of biomechanical factor in the pathogenesis of the condition.

Conclusion All tested eye tissues are characterized by nonlinear age dynamics of biomechanical properties, which must be taken into consideration in biomechanical studies of eye pathology.

■ 2157

Biomechanical analysis of the hypothesis on "rest of accommodation"

KOSHITZ IN (1), SVETLOVA OV (2), SMOLNIKOV BA (3), GORBAN AI (4), KOTLIAR KE (5)

(1) Petercom / MS Consulting Group Cl. corp, St.Petersburg

(2) Department of Ophthalmology, Medical Academy of Postgraduate Education, St.Petersburg

(3) Department of Mechanics and Control Processes, Polytechnic University, St.Petersburg

(4) -, Aachen

(5) Department of Ophthalmology, Munich University of Technology, Munich

Purpose In his accommodation theory Helmholtz (1855) introduced two concepts: „accommodation“ and „no accommodation“. Later the concept of “the rest of accommodation” was introduced by some authors on the base of experimental studies (e.g. Mütze, 1956). This concept describes an intermediate state of ciliary muscle (CM) between near-sight and far-sight. Additionally, according to latter concept a higher tonus of CM corresponds to the near- and far-sight accommodation. Gorban (2005) showed essential disadvantages of experiments by Mütze and argue away the concept of “the rest of accommodation” on the base of these experiments.

Methods Using control theory and based on the Mechanics laws the detailed analysis of clinical and experimental data on accommodation was performed.

Results Only in the positional pre-adjustment the accommodation system of the eye is able to re-tune quickly the system of visual perception in waking ours and to save the energy during prolonged functioning of CM at the near sight.

Conclusion Traditionally accepted state of “rest of accommodation” does not exist during waking hours. Though there is a state of active pre-adjustment of the accommodation system of the eye. In this state the energy consumption of CM is not minimal but it is optimized for a quick response to the appearance of a visual stimulus. “Complete near sight” characterizes the state of accommodation system with maximal CM-tonus and its’ maximal energy consumption, while “complete far sight” corresponds to the minimal CM-tonus.

■ 2161

Quantification of Drusen Deposits using Image Processing Techniques

MORA A (1, 2), FONSECA J (1, 2), VIEIRA P (2)

(1) Intelligent Robotics Center, Uninova, Caparica

(2) Faculty of Sciences and Technologies, Universidade Nova de Lisboa, Lisbon

Purpose The automatic analysis of Drusens quantitative evolution can be a useful tool for ophthalmologists that are currently analyzing it visually in a not reproducible process. In this paper we propose a methodology for automatic quantification of Drusens in retina images using image processing techniques.

Methods Often, these images have a non-uniform illumination that produces different contrast areas. In this work we propose an algorithm that compensates the image non-uniform illumination. In a second step we propose an innovative approach for Drusens detection and quantification. It consists in the creation of a tri-dimensional model of each Drusen spot, allowing areas and estimated volumes to be computed. The algorithm starts by localizing spots and determining their influence areas. Finally, using the image intensity as tri-dimensional information, the spots detected on the previous step are modeled using tri-dimensional functions.

Results The results of applying the methodology to retina images compared with images marked by ophthalmologists are presented. The comparing images were marked through a semi-automatic procedure in an application specifically developed for this purpose that is now being used on clinical practice.

Conclusion We present a new methodology for quantitative analysis of Drusens on retina images that is reproducible and independent of visualization conditions. This software will allow quantitative studies about Drusens evolution along treatments to be elaborated.

■ 2162

Early neuroglial loss in DM type 1 patients, an OCT study

VERBRAAK FD (1, 2), BIALLOSTERSKI C (1), VAN VEITHOVEN ME (1),

SCHLINGEMANN RO (1), MICHELS BPJ (3), DE VRIES HH (3)

(1) Academic Medical Center, Dept of Ophthalmology, Amsterdam

(2) Academic Medical Center, Laser Center, Amsterdam

(3) Academic Medical Center, Dept of Internat Medicine, Amsterdam

Purpose To confirm the hypothesis of early neuroglial loss in diabetic retinopathy (DR) we compared retinal thickness (RT), measured with Optical Coherence Tomography (OCT) in patients with type 1 diabetes (DM), with no or minimal DR, to healthy controls.

Methods Fifty-three patients with type 1 DM with no or minimal DR, seen at the outpatient clinic of the department of internal medicine at the AMC, underwent full ophthalmic examination, fundus photography and OCT. Mean RT was calculated for the central fovea, the fovea, the pericentral and the peripheral area of the macula and compared to normal controls.

Results Mean RT in the pericentral area was lower in patients with minimal DR ($267\mu\text{m} \pm 20\mu\text{m}$; n=23, 43%) compared to healthy subjects ($281\mu\text{m} \pm 13\mu\text{m}$; P value = 0.005, 95% CI = -23.10, -4.46; n=28). The mean pericentral RT in patients without DR ($276\mu\text{m} \pm 14\mu\text{m}$; n=30, 57%) was less than pericentral RT in normal controls, but more than in patients with minimal DR, but did not differ significantly from either one. None of the other regions showed a significant change.

Conclusion In this study a significantly decreased pericentral RT was measured in patients with minimal DR compared to healthy controls. This could be explained by a loss of intraretinal neural tissue in the earliest stage of DR.

■ 2163

Establishment of a normative database by registration of RTA maps into the RT-Atlas

BAPTISTA P (1), SANTOS T (1), NUNES S (1), BERNARDES R (1), CUNHA-VAZ J (1, 2, 3)

(1) AIBILI - Association for Innovation and Biomedical Research on Light and Image, Coimbra

(2) Center of Ophthalmology, Coimbra University Hospital, Coimbra

(3) Faculty of Medicine, University of Coimbra, Coimbra

Purpose To establish an improved retinal thickness normative database.

Methods Retinal thickness measurements by RTA II (Retinal Thickness Analyzer, Talia, Israel) were obtained from 27 eyes from 20 healthy volunteers aged from 40 to 64 years (mean \pm SD: 53.3 \pm 5.9 yrs). Each retinal thickness map was registered into the retinal thickness atlas (RT-Atlas) by considering translations and rotation, thus becoming into alignment with the RT-Atlas space, where the horizontal line connects the center of the fovea to the center of the optic disc and the fovea is at the origin of the coordinates system. While the RT-Atlas is established using principal components analysis, this normative database is established at the RT-Atlas space coordinates by thin-plate spline interpolation to compute the average, standard deviation (SD) and the 95% confidence interval (CI).

Results Values for the established retinal thickness normative database range from 147.1 μm (95% CI = [139.0-155.2]) to 219.1 μm (95% CI = [211.8-226.4]), while the standard deviation range from 13.9 μm to 24.1 μm (SD/mean from 6.8% to 14.8%).

Conclusion The established normative database in association to the registration of thickness maps into the RT-Atlas space, allows to better identify retinal thickness changes from the control healthy population and to better follow retinal changes over time.

■ 2164

Increased OCT Mapping Resolution by Merging Line and Circle Scan-Types

SANTOS T (1), BAPTISTA P (1), BERNARDES R (1), CUNHA-VAZ J (1, 2, 3)

(1) AIBILI - Association for Innovation and Biomedical Research on Light and Image, Coimbra

(2) Center of Ophthalmology, Coimbra University Hospital, Coimbra

(3) Faculty of Medicine, University of Coimbra, Coimbra

Purpose To increase retinal thickness mapping resolution of the Optical Coherence Tomograph (OCT - Humphrey-Zeiss, USA).

Methods A retinal thickness Atlas (RT-Atlas) of the human macula was established based on a series of 33 retinal thickness maps from the Retinal Thickness Analyzer (RTA II, Talia, Israel), from 20 healthy volunteers. A principal component analysis was performed to extract the major retinal thickness characteristics to build the RT-Atlas. Two scan-types were performed using the Stratus OCT: a radial scan set and a circular scan set. The radial scan set (six-radial scans - fast scan mode) are initially registered to the RT-Atlas by an iterative optimization procedure to compute the global position and orientation for the entire set, followed by individually finding the best fit of each line-scan in the neighbourhood of the computed global parameters. A new retinal thickness map is then built based on the OCT data (now registered to the RT-Atlas, thus having an origin centered on the fovea and the horizontal axis pointing to the center of the optic disc). The set of circular scans (fast scan mode) is now registered into this new map following a similar procedure, i.e., global and individual fits, thus achieving the registration of both radial and circular scans.

Results This method allows for the registration of radial and circular OCT scans to achieve an increased retinal thickness mapping resolution, and was applied to both healthy and diseased retinas.

Conclusion This new map presents an increased resolution compared to the original OCT mapping and proved to be useful for detecting small retinal thickness changes.

■ 2165**May OCT/SLO improve our understanding in pathophysiology of optic disc coloboma-related maculopathy?**

PICCIIRILLO V (1), RAO V (2), DI NUZZO A (2), SAVASTANO S (1), SAVASTANO MC (3), FORTE R (4), MOSCATO B (5), ROSEN R (6)
 (1) Eye Department, Civilian Hospital Santa Scolastica, Cassino
 (2) Eye Department, Civilian Hospital of Maddaloni, Maddaloni
 (3) Eye Department, Catholic University, Rome
 (4) Eye Department, University of Naples Federico II, Naples
 (5) Radiology Department, Civilian Hospital G. Rummo, Benevento
 (6) Eye Department, New York Eye and Ear Infirmary, New York

Purpose To describe OCT-SLO findings in a case of unilateral optic disc coloboma complicated by serous macular detachment and macular lamellar hole.

Methods A young white woman (34 yo) presented at our Primary Care Unit with a 1 month history of decreased vision and metamorphopsias in her left eye. Family history was negative for any ophthalmic disorder. Complete ophthalmic examination, colour fundus photos and OCT-SLO were performed.

Results Best corrected visual acuity: 20/20 in the right eye, 20/200 in the left eye. IOP: 17 mmHg in both eyes. Fundus examination of the left eye revealed an optic disc coloboma with pigmentary changes in peripapillary temporal area. A serous macular detachment with an oval full thickness macular hole-like lesion were observed. The OCT-SLO evaluation showed a large schisis-like serous macular detachment and partial thickness macular hole; the optically empty space within retinal layers appeared to be continuous with optic disc coloboma. The coronal imaging of optic disc revealed an eccentric, optically empty depression covered by an unbroken thin layer of neurosensory retinal-like tissue, extending underneath the RPE; on the bottom, a small, oval shaped, cystic space, contiguous to the coloboma's wall, was observed on the infero-temporal side suggesting the subretinal-subarachnoidal interspaces communication.

Conclusion Macular schisis is the leading cause of visual loss in case of "complicated" optic disc coloboma. Although the origin of sub retinal fluid remains unclear, OCT SLO technique demonstrates the continuity between sub retinal fluid and the coloboma suggesting the possible subarachnoidal source of the maculopathy.

■ 2166**Development of a Fluorescent Lifetime Image Ophthalmoscope**

BARBOSA A (1), MANIVANNAN A (2), VIEIRA P (1)
 (1) Departamento de Física, Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, Lisbon
 (2) Department of Biomedical Physics and Bioengineering, University of Aberdeen, Aberdeen

Purpose Development of a fluorescence lifetime image ophthalmoscope is presented. The main goal is to retrieve an image of the retina based on its fluorescent response, both intensity and lifetime, and use the Stern-Volmer correlation to map oxygenation levels.

Methods Melanin and lipofuscin are two molecules existent in the retina that exhibit fluorescence when stimulated with light at specific wavelengths and information about their concentration and chemical environment, namely the presence of oxygen (quenching), can be retrieved. Fluorescence lifetime was calculated by measuring the phase shift between the fluorescence response and a high frequency modulated light source. The advantage of this technique when compared to others is the use of low exposition times to the exciting radiation and the use of low power light sources, making it compatible with in-vivo imaging. Clinical applications include early diagnosis of age related macular degeneration (ARMD).

Results In this work a modulated laser beam at a fixed wavelength (440nm) was used as excitation source, a 200 MHz lock-in amplifier was used to provide the modulation frequencies and phase measurement and an avalanche photodiode to detect the fluorescence emission. Fluorescent solutions with different lifetimes were used to test and calibrate the system.

Conclusion Preliminary results indicate that this is a potential technique to image the human fundus since it allows using of low power radiation, low exposition times and does accurate phase measurement even with high noise to signal ratios. Integration of this system in a scanning laser ophthalmoscope prototype was made to obtain 2D images of the samples, showing very promising results of the potential of this technique for ophthalmologic application.

■ 2167**Establishment of a Retinal Thickness Atlas (RT-Atlas)**

BERNARDES R (1), SANTOS T (1), CUNHA-VAZ J (1, 2, 3)
 (1) AIBILI - Association for Innovation and Biomedical Research 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 establish a retinal thickness atlas (RT-Atlas) of the human eye.

Methods The RTA II (Retinal Thickness Analyzer, Talia Technology, Lod Indst Area, Israel) was used in this work to collect retinal thickness information from a set of 33 retinas (14 right-eyes and 19 left-eyes) of 20 healthy volunteers aged from 40 to 64 years-old (mean \pm SD: 52.4 \pm 5.9). Each of these 33 retinal thickness maps was aligned and rotated to correct for the location and orientation (left eyes were flipped vertically), being the horizontal axis considered the line connecting the center of the fovea to the center of the optic disc. Principal component analysis was applied to this stack of information to capture the major characteristics of the retinal thickness information in a set of eigenthicknesses. The final RT-Atlas is the eigenthickness associated to the highest eigenvalue, thus retaining most of the energy of the entire ensemble.

Results The RT-Atlas established allows registering different retinal thickness measurements to the model using the same or different techniques, e.g., optical coherence tomography, using thickness information only and therefore to correct for the location and rotation of the different maps even in the absence of fundus references.

Conclusion The RT-Atlas established in this work corresponds to an expectation model for the retinal thickness in retinas of healthy individual and allows for the registration of different retinal thickness measurement techniques to the same reference, e.g., RTA and OCT.

■ 2211**Risk factors for hypermetropia, strabismus and amblyopia in the ALSPAC Study**

WILLIAMS C (1), NORTHSTONE K (2), HOWARD M (3), HARRAD RA (1), SPARROW JM (1)

(1) *Ophthalmology, Bristol*

(2) *Community Based Medicine, University of Bristol, Bristol*

(3) *Orthoptics, Bristol*

Purpose Moderate/high hypermetropia, strabismus and amblyopia are common problems for children in many countries. Data on prevalence and risk factors are important for planning service provision and potentially for devising preventative strategies.

Methods Data were obtained from the Avon Longitudinal Study of Parents and Children (ALSPAC). Over 7000 children attended a visual assessment when they were aged 7 years and were examined by an orthoptist who used an autorefractor to estimate refractive error. Prospectively collected background data regarding family history of vision problems, demographic, socio-economic and early life factors were available from ALSPAC.

Results Of 7538 children, 10.0% (9.4% – 10.8%) were at least +1.00 or more hypermetropic; 3.6% (3.3% – 4.1%) had clinically significant convergent strabismus and 3.6% (3.3% - 4.1%) had past or present amblyopia. The prevalence of hypermetropia was 2.8% in children from social class I vs 7.2% in children from social class IV/V ($p < 0.001$). Hypermetropia was also associated with type of housing the family lived in ($p = 0.003$) and with whether or not the child was breast-fed ($p < 0.001$). The risk factors for convergent strabismus and for amblyopia were similar, but those for divergent strabismus were different; only gestation ($p < 0.001$) and maternal smoking in pregnancy ($p = 0.011$) were predictive.

Conclusion Hypermetropia and related problems are common in this population at 7 years. Environmental, as well as genetic factors appear to play an important part in determining whether a child is hypermetropic, or strabismic at this age. These data will help plan clinical care provision and may help in the design of preventative strategies.

■ 2212**Comparisons of refractive error in UK and Singapore children: the ALSPAC and SCORM studies**

GAZZARD G (1), WILLIAMS C (2), SAW S-M (3), MILLER L (2), ALSPAC STUDY GROUP (2)

(1) *Institute of Ophthalmology, London*

(2) *Dept of Community-Based Medicine, University of Bristol, Bristol*

(3) *Dept of Community, Occupational and Family Medicine, National University of Singapore, Singapore*

Purpose To compare risk factors for myopia in English and Singaporean children

Methods Refraction at age 7 was obtained in two population cohorts: the Singapore Cohort Study of Risk Factors for Myopia (SCORM; n = 840, cycloplegic refraction) & the Avon Longitudinal Study of Parents and Children, UK (ALSPAC; n = 6758, non-cycloplegic refraction).

Results Myopia >0.5D or greater was found in 13.6% (95% CI 12.8 to 14.4) in ALSPAC and 25.7% (22.7 to 28.7) in SCORM. In both populations the number of myopic parents was the main predictor of myopic status: adjusted OR 1.32 (95% CI 1.14, 1.52; $p < 0.001$) for 1 parent & (1.50 (1.14, 1.97; $p = 0.004$) for 2 parents in ALSPAC and 1.59 (1.06, 2.36; $p = 0.024$) & 3.22 (2.05, 5.08; $p < 0.001$) in SCORM. There was a non-significant trend for Asian children to have a higher risk for myopia than white children in the Avon data. In the SCORM data there was no difference in risk between Indian vs. non-Indian Asians ($p = 0.855$). Sex was not a significant predictor in either. Parental education was not a significant predictor of myopia in ALSPAC ($p = 0.89$). In SCORM the univariate risk with tertiary vs. secondary paternal education was 1.60 (1.16, 2.20; $p = 0.004$), but in adjusted analyses was 1.08 (0.76, 1.55; $p = 0.66$). A preliminary pooled analysis of SCORM & ALSPAC Asians data suggested country of residence was not a significant risk factor for myopia.

Conclusion Number of myopic parents was the main predictor of myopic status at age 7 in both populations. Sex, ethnicity and parental education had little or no influence. Further exploration of the similarities and differences in these two cohorts may help to determine the mechanisms involved, particularly of gene-environment interactions.

■ 2213**Underlying patterns of refractive development in Sydney children: the Sydney Myopia Study**

MORGAN IG (1), ROSE KA (2)

(1) *ARC Centre of Excellence in Vision Science, Australian National University, Canberra*

(2) *Faculty of Health Sciences, University of Sydney, Sydney*

Purpose To assess the concept of emmetropisation, commonly viewed as a mechanism for feedback control of eye growth to achieve and maintain normal vision.

Methods Cycloplegic spherical equivalent (SE) measures (1% cyclopentolate) and corneal radius of curvature (CR) and axial length (AL) were obtained on two cross-sectional samples of 1749 Year 1 and 2353 Year 7 students from a random sample of schools in Sydney.

Results The kurtosis of the distribution of SE was high in the Year 1 sample, and was similar in the Year 7 sample. CR was identical in the two samples, but there was an increase in AL. Plots of SE against the ratio of AL to CR (a measure of eye geometry) were triphasic. The hyperopic and myopic arms were steep, with SE decreasing by around 2.7D/mm of AL. In the intermediate zone, SE decreased by only 0.5D/mm.

Conclusion These data define three phases of refractive development. In the first, active matching of AL to CR rapidly reduces refractive errors and leads to a highly leptokurtotic distribution of SE. This process may be complete within the first two years of life. In the second, myopic shifts due to increases in AL are neutralised by decreases in lens power. This phase appears to prevent emmetropisation, instead producing a preferred state of mild hyperopia in which vision can be cleared by accommodation. It reduces the myopic shift during development by as much as 4D. In the third, further increases in AL lead to myopic shifts without compensating mechanisms. This explains the abrupt onset of rapid myopic progression. How rapidly children pass through the latter two phases depends on the rate of increase in AL, which may be predominantly determined by environmental exposures. Neither of these phases corresponds to emmetropisation.

■ 2214**Prevalence and risk factors for myopia, hypermetropia, strabismus and amblyopia in Australian children: the Sydney Myopia Study**

ROSE KA (1), MORGAN IG (2)

(1) *School of Applied Vision Sciences, University of Sydney, Sydney*

(2) *ARC Centre of Excellence in Vision Science, Australian National University, Canberra*

Purpose To examine prevalence and risk factors for refractive error, and associations with strabismus and amblyopia.

Methods Two cross-sectional samples of 1749 Year 1 and 2353 Year 7 students from a random sample of 51 schools in Sydney, participated in a comprehensive eye examination including cycloplegic (cyclopentolate 1%) autorefraction, LogMAR visual acuity and cover test. Families completed extensive questionnaires on health, medical, lifestyle and socio-demographic factors.

Results The prevalence of myopia (SE<-0.5D) in Year 1 students (mean age 6.7 years) was 1.5% and in the Year 7 students (mean age 12.7 years) it was 12.8%. In the Year 7 students, myopia was significantly associated with parental myopia and East Asian ethnicity ($p < 0.0001$). Year 7 students who engaged in the lowest tertile of hours spent in outdoor activity and highest tertile of near-work had an odds ratio of 2.64 for myopia compared to those children doing the lowest tertile of outdoor activity and highest tertile of near-work. The prevalence of hyperopia (SE>+2.0D) in Year 1 students was 13.0% and 5.0% in the Year 7 students. Strabismus in the Year 1 students (2.8%) was significantly associated with amblyopia, anisometropia, astigmatism and hyperopic refractive error ($p < 0.0001$) and with low birthweight and admission to a neonatal intensive care unit ($p < 0.01$).

Conclusion The prevalence of myopic refractive error is low in Australian school children by international standards. There was a significant association between high levels of outdoor activity and a more hyperopic refractive error. Strabismus and amblyopia were significantly associated with adverse perinatal health and refractive errors.

■ 2215

**The epidemiology of refractive error in UK children:
the Aston Eye Study**

LOGAN NS (1), SHAH P (1), RUDNICKA AR (2), OWEN CG (2), GILMARTIN B (1)

(1) School of Life & Health Sciences, Aston University, Birmingham

(2) Division of Community Health Sciences, St George's, University of London, London

Purpose The Aston Eye Study (AES) is a 3-year prospective cross-sectional study (started October 2005) to determine the prevalence of refractive error and to describe ocular biometry in a large multi-racial sample of school children from the West Midlands (England).

Methods Schools are selected by random sampling within the West Midlands area, stratified by area deprivation index (a measure of socio-economic status). Schools with pupils predominantly from a single race are excluded. Sample size calculations account for the likely participation rate and the clustering of individuals within schools. Two separate age groups are being sampled: 1700 Year 2 children (age 6/7 years) and 1200 Year 8 children (age 12/13 years). Visual acuity, non-contact ocular biometry (axial length, corneal radius of curvature and anterior chamber depth using IOLMaster Zeiss, Jena) and cycloplegic (proxymetacaine 0.5%:cyclopentolate 1%) open-field autorefraction (Shin-Nippon SRW5000, Japan) are measured in both eyes. Oculomotor balance (cover test), height and weight are also assessed. Questionnaires for parents and older children will allow the influence of environmental factors on refractive error to be examined.

Results The initial stages of data collection (N=226) and protocols have been well received by both parents and children. No adverse incidents or effects have been reported to date. Currently almost 1 in 15 children require refractive correction but do not have the provision of spectacles.

Conclusion The AES will allow the ocular characteristics of 2900 children from a large metropolitan area of England to be described. The association between educational status, ethnic background and other environmental influences on refractive outcome will be determined.

■ 2221

What is new in endogenous endophthalmitis ?

BODAGHI B

Ophthalmology, University of Paris VI, Paris

Purpose Endogenous endophthalmitis (EE) remains a challenging issue for ophthalmologists. Clinical manifestations may be less impressive than those encountered in cases of post-surgical acute endophthalmitis and patients may present with an inflammatory eye masquerading as an acute-onset uveitis. This important characteristic leads to a delayed diagnosis and a classical poor visual outcome. Identification of the primary source of infection remains a priority. Diagnostic and therapeutic management of patients with EE have changed during the last few years. Diabetes, intravenous drug abuse and recent gastrointestinal surgery are major risk factors. Injection of buprenorphine contaminated with oral Candida species is one of the commonest risk factors encountered in patients with fungal endophthalmitis. The use of molecular tools such as PCR and culture applied to ocular fluids enables us to isolate the microbial agent earlier. Technical difficulties on the proper handling of the specimens should still be resolved. Therapeutic management of patients with EE is based on the early use of a well-adapted anti-microbial regimen. New molecules are available for specific local or systemic treatment. The optimal use of corticosteroids is still controversial in this condition.

■ 2222

Relationship between bacterial adhesion to intraocular lenses and postoperative endophthalmitis

KODJIKIAN L

Dept of Ophthalmology, Croix-Rousse Hospital, Lyon

Purpose Postoperative endophthalmitis following intraocular lens (IOL) implantation is still one of the most feared complications of cataract surgery. Bacterial adhesion to IOLs during their insertion is a prominent etiological factor. Polypropylene was the first biomaterial that allowed this relation of cause and effect to be proven. Following adhesion, bacteria replicate, congregate and form multiple layers of microcolonies which actually represent the basic structural unit of the biofilm. The bacteria are embedded in a slime layer. Adhesion to IOLs has been studied by several in vitro studies and discrepancies can be found between them which are due to variations of experimental conditions. The strains, the incubation times and the methods all varied. Adhesion is affected by the nature of the IOLs, the isolates and the surrounding medium. Since this medium is very difficult to model because of its complexity, in vivo studies seemed essential. We have recently determined in vivo evolution of the amount of attached bacteria to five types of IOLs. Moreover, we recently developed a novel in vitro model to study not only bacterial adhesion but also bacterial colonisation. There have been few epidemiological studies published to determine the relationship between endophthalmitis and the IOL type. However, the perfect biomaterial that could prevent postoperative endophthalmitis does not yet exist. Globally, hydrophilic materials and hydrophobic acrylic seem to be less sticky than silicone or PMMA, but this remains to be proven clinically.

■ 2223

Endophthalmitis after cataract surgery, risk factors and prophylaxis : myths and facts

GARWEG JG (1), KODJIKIAN L (2)

(1) *Ophthalmology, Bern*(2) *Ophthalmology, Lyon*

Purpose Acute postoperative endophthalmitis (POE) is still the most serious complication of cataract surgery.

Methods Several studies have reported a reduction in the incidence of POE following the change from intra- to extra-capsular extraction, but no further reduction in incidence has been reported since the change from extracapsular extraction to phacoemulsification. Incidence appears to have even increased over the past decade.

Results Possible risk factors for this tendency include the surgical trauma itself, incision construction, the IOL material and surface composition, and (thanks to a less invasive surgical technique) an increased systemic risk profile. Sutureless clear corneal incision for phacoemulsification has eventually been the most relevant change in the surgical technique relying on postoperative patient cooperation. Nevertheless, the implant will remain the most significant source for bacterial invasion. Therefore, most recent clinical and experimental prophylaxis studies have namely focussed on the IOL material and surface composition and on antibiotic delivery to the eye. Based on published scientific evidence the most effective prophylaxis of POE is a lavage of the conjunctival sac using povidone-iodine 5% for 90 seconds at the begin of surgery. Moreover, the recently published ESCRS European prospective randomized multicenter study undoubtedly demonstrated that intracameral cefuroxime (1 mg) administered at the time of surgery significantly reduced the risk to develop endophthalmitis after cataract surgery.

Conclusion The combination of preoperative povidone-iodine and intraoperative cefuroxime evolves to be the current evidence-supported standard for POE prophylaxis after cataract surgery.

■ 2224

Endoscopy for the management of severe endophthalmitis

DE SMET M (1, 2), GHYCZY-CARBORG EAE (2), MUURA M (2)

(1) *Division of Vitreo-retinal and Uveitis, Department of Ophthalmology, ZNA Middelheim, Antwerp*(2) *Department of Ophthalmology, Academic Medical Center, University of Amsterdam, Amsterdam*

Purpose In severe endophthalmitis, visualization of intraocular structures is severely limited due to the presence of corneal edema/infection, fibrin or pus in the anterior chamber, clouding of the lens or lens capsule. While the anterior segment can be mechanically cleared in many cases at the onset of pars plana vitrectomy, some cases for adequate management may require a keratoprosthesis. Endoscopy bypasses visualization constraints placed by the anterior segment during pars plana vitrectomy. This study investigates the results of patients with severe endophthalmitis managed by endoscopic pars plana vitrectomy.

Methods Retrospective consecutive study of endophthalmitis cases managed using an endoscope. State on anterior segment, etiology of infection, vision and ocular outcome at 6 months, and final follow-up were recorded. Three port vitrectomy was carried out with an endoscope. Vitreous was as much as possible removed, as were antero-posterior and equatorial traction bands. State of the retina was investigated. Intravitreal antibiotics were administered. In a follow-up study, in retinal necrosis patients, silicone oil was used.

Results Of 15 patients initially treated using this approach, 8 retained CF vision or more. Six patients had 0.2 or more vision. Enucleation was required in 4 patients. In a subsequent study using silicone oil in patients with retinal necrosis, all 4 patients did not require enucleation.

Conclusion Endoscopy is a valuable alternative in the management of severe endophthalmitis which allows rapid staging of the ocular condition, extensive debridement of the infected tissue, and implementation of an appropriate strategy for eye salvage and where possible recovery of vision.

■ 2225

New antibiotics in endophthalmitis treatment

COCHEREAU^I

Ophthalmology, Angers

Purpose In endophthalmitis, intravitreal antibiotics are mandatory, topical antibiotics are optional and systemic antibiotics remain controversial. Since 2000, only one new antibiotic (linezolid) has been available. Despite its broad spectrum activity against Gram positive bacteria (GPB), it has not been yet evaluated in endophthalmitis. Intravitreal antibiotics are the reference therapy even if none of them has been officially approved and if no large randomised studies have been published. Due to the diversity of causative bacteria, a combination of two antibiotics is recommended: vancomycin still efficient against GPB, and either amikacin with its risk of macular toxicity, or ceftazidime with its risk of intravitreal precipitation. Intravitreal fluoroquinolones have few interests due to the increasing rate of resistance. Intravitreal injection must be realized as soon as possible, even if no operating room is readily available. Intravitreal steroids may be associated as soon as the infection is controlled. Repeated intravitreal injections are limited by the ocular toxicity. Laterobulbar injections of antibiotics are no longer used. Topical antibiotics are indicated to treat a superficial abscess, but they do not kill intraocular micro-organisms. Systemic therapy is controversial. The addictive effect of intravenous ceftazidime–amikacin to intravitreal therapy has not been demonstrated in the EVS, but these two drugs were not of choice due to their poor intravitreal kinetics and low antistaphylococcal efficacy. The interest of systemic antibiotics with more favourable kinetics like imipenem or moxifloxacin remains to be demonstrated. In ophthalmology, a good use of antibiotics is mandatory to preserve antimicrobial efficacy in severe infections.

■ 2226

Complete and Early Vitrectomy for Eyes with Postoperative Endophthalmitis

KUHN F, GINIG

, Birmingham, USA

Purpose Following recommendations in the Endophthalmitis Vitrectomy Study (EVS), vitrectomy for postoperative endophthalmitis is performed today only if visual acuity drops to light perception. The authors evaluated their results achieved by performing complete and early vitrectomy for these eyes.

Methods The authors treat their patients with postoperative endophthalmitis very differently. If there is excellent red reflex or some retinal details are visible, intravitreal antibiotics and corticosteroids are injected, and the patient is closely monitored for 24 hours. If the condition does not improve or if there is no red reflex/retinal visibility, complete vitrectomy is performed, regardless of the visual acuity. Every effort is made to remove the posterior hyaloid and clean the macular area.

Results In a consecutive series of 47 patients, we achieved 0.5 or greater vision in 91% of eyes with no case of retinal detachment, phthisis, or enucleation. These results are significantly better than those seen in the EVS.

Conclusion The authors advocate complete and early vitrectomy for eyes with postoperative endophthalmitis. Complications of both the purulent process and its therapy are reduced if the surgical intervention is early and aimed at total removal of the infectious and inflammatory debris. Both the anatomical and functional results have been found to be superior to those achieved with conservative therapy.

■ 2231**Contribution of the Eye-Associated Lymphoid Tissue (EALT) to immune regulation at the ocular surface**

KNOPE (1), KNOPE (2)

(1) Research laboratory, Eye Clinic, Charite-Universitätmedizin Berlin, Berlin

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

Purpose The immunologic homeostasis at the mucosal surfaces is governed by mechanisms of the so called mucosal immune system. This also appears to apply to the mucosal surface of the outer eye where an Eye-associated lymphoid tissue (EALT) is described with common characteristics as in other tissues.

Methods Own results on the mucosal immune system of the human ocular surface are introduced and discussed together with results from a Medline based literature recherche.

Results Recent results show, that in addition to innate immune factors, the normal human ocular surface also contains components of the specific mucosal immune system. The afferent arm of mucosal immunity is represented by lymphoid follicles for antigen presentation and effector cell generation in the conjunctiva and lacrimal drainage system. A diffuse lymphoid tissue composed of the respective effector cells (lymphocytes and plasma cells) that is continuous from the lacrimal gland along the conjunctiva and the lacrimal drainage system forms the efferent arm. Resident lymphatic cells of the mucosal immune system have the task to maintain immune protection of the ocular surface by favoring immune tolerance and the secretion of anti-inflammatory IgA antibodies. In different inflammatory diseases of the ocular surface, however, (as e.g. the dry eye syndrome, ocular allergy) there is a T-lymphocyte mediated inflammation that represents a deregulation of the mucosal immune system.

Conclusion Eye-associated lymphoid tissue, formed by physiologic protective resident lymphocyte populations, represents a part of the mucosal immune system of the body and regulates the immunologic homeostasis at the ocular surface.

■ 2232**Role of M-cells in the afferent limb of ocular surface immunity**

KNOP N (1), KNOP E (2)

(1) Dept. of Cell Biology in Anatomy, Medical School Hannover, Hannover

(2) Research Lab., Dept. of Ophthalmology, Charité, Berlin

Purpose Follicular M-cells are an important part of the afferent arm of mucosal immunity for the uptake and transport of luminal antigens. Lymphoid follicles also occur in the conjunctiva-associated lymphoid tissue (CALT) which is a part of the eye-associated lymphoid tissue (EALT) at the ocular surface. There is some evidence in the human and they occur more prominently in the rabbit.

Methods Flat whole-mount conjunctival tissues from the human (54) and the rabbit (42) were investigated in cleared whole-mounts, in paraffin histology, scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Results Lymphoid follicles occur in a majority of human conjunctivas in smaller numbers but are a prominent characteristic in the rabbit where they accumulate into a dense group nasally towards the lacrimal punctum of the lower lid. These have typical characteristics as high endothelial venules (HEV) and bright germinal centers (GC) with lymphoblasts, follicular dendritic cells, apoptotic cells and tingible body macrophages. The follicle-associated epithelium (FAE) contains groups of lymphoid cells in both species. In the rabbit TEM shows them to be located in cytoplasmic pockets of superficial cells with a thin cytoplasmic luminal lining. The M-cells are located between and on top of the ordinary dense epithelial cells. In SEM, these surface cells are very large, have a polygonal outline and cover cavernous spaces.

Conclusion Lymphoid follicles in the human occur physiologically but appear inconspicuous. In the rabbit they are more prominent and it can be observed that CALT has a typical follicular morphology including HEV for regulated lymphocyte migration and epithelial cells with ultrastructural characteristics of M-cells. The fact that these M-cells indeed allow a transport of luminal antigens is indicated by the formation of germinal centers of proliferating lymphocytes in the underlying follicles.

■ 2233**Aspects of innate immunity at the ocular surface**

DUA HS, ABEDIN A, RAHMAN I, MATHEW M, HOPKINSON A

Ophthalmology and Visual Sciences, Nottingham

Purpose The ocular surface is constantly exposed to environmental pathogens and consequently has developed a multitude of defences, both humoral and cellular. We present aspects of innate immunity at the ocular surface in relation to antimicrobial peptides, toll like receptors and immune cell migration.

Methods RNA was extracted from ocular surface epithelial cells obtained by impression cytology of normal and diseased eyes (bacterial, viral, acanthamoeba, dry eyes) and from cultured cells. RT-PCR for TLRs 1-10 and 21 antimicrobial peptides (AMP) (HBD1 to DEFB 129) was performed. Organ cultured human conjunctival specimen, denuded of the epithelium were examined for trans-basement membrane immune cell migration. Cells were studied by EM, immunohistology and FACS.

Results Human beta defensins 1 and 2 were expressed in all samples, HBD3 in some samples but not in controls. HBD4 was found rarely in bacterial and acanthamoeba samples but not in the others. Liver expressed antimicrobial peptides LEAP-1 and -2 and LL37 were also found in most samples though the profile varied. A novel antimicrobial peptide, RNase 7, a basic skin protein with a broad antimicrobial spectrum was detected in all samples. All TLRs except TLR9 were widely expressed. TLR 10 was also noted for the first time. CD4+ T cells, NK cells and CD8+ T cells were the predominant migrating cells in the first 72 hours. Thereafter only NK cells were detected. B cells and HML-1+ cells constituted a small but significant proportion.

Conclusion The ocular surface has a complex innate and adaptive defence repertoire. A wide range of AMPs and TLRs are expressed. The AMPs, besides having direct antimicrobial effects also trigger adaptive immune responses via release of cytokines and recruitment of specific cells.

■ 2234**The Role of Surfactant Protein-D (SP-D) at the Ocular Surface**

BRON AJ (1), CLARK HW (2)

(1) Nuffield Laboratory of Ophthalmology, Oxford

(2) MRC Immunobiology Unit, University of Oxford, Oxford

Purpose To review the role of SP-D at the ocular surface.

Methods A literature review.

Results SP-D belongs to the family of proteins termed collectins, which possess both collagenous and lectin-binding (carbohydrate recognition) domains. They play a role in the innate response to allergens and microbial pathogens by binding sugars at the surface of certain microorganisms, in a calcium-dependent manner, inhibiting cell invasion and facilitating clearance by scavenging macrophages. They are constitutively expressed by many cells and their expression may be up-regulated by microbial challenge. Surfactant protein-D (SP-D) is a ubiquitous protein found in the lung and at non-pulmonary mucosal surfaces including the eye. It is expressed in the lacrimal gland and ducts, the cornea and conjunctiva and is found in the tears in moderate concentration. The presence of SP-D in tears explains in part their ability to inhibit corneal epithelial cell invasion by certain strains of *Ps. aeruginosa* (PAK). SP-D is expressed constitutively by human and mouse corneal epithelium and is up-regulated by microbial challenge (*Ps. aeruginosa*): corneal epithelial invasion by *Ps. aeruginosa* is enhanced in SP-D knockout mice and blocked by recombinant human and mouse SP-D. SP-D also binds to the glycoprotein GP-340 or DMBT-1, a normal tear constituent of the gel phase of the tears. GP-340 is also expressed by cell membranes and is a putative ligand for the uptake of SP-D, complexed with microorganisms, into macrophages.

Conclusion SP-D is an important component of the innate response of the ocular surface to microbial challenge.

■ 2235**Flow cytometry study of Impression Cytology specimens during the long term follow-up of severe ocular burns**

GICQUEL JJ (1), NAVARRE R (1), GOMBERT JM (2), DIGHIERO PL (1)

(1) Jean Bernard University Hospital / Ophthalmology Department, Poitiers

(2) Jean Bernard University Hospital / Pathology Department, Poitiers

Purpose To evaluate the usefulness of impression cytology (IC) in the long-term follow-up of severe ocular alkali burns.

Methods Twenty six patients (30 eyes) with severe alkali burns (Grade III of Dua classification or higher) were referred to our department of ophthalmology and enrolled prospectively. Amniotic membrane transplantation (AMT) was performed in all patients before day two. IC specimens were collected for flow-cytometry analysis in all patients before AMT, after 2, 6, 12, 18, and 24 months. HLA-DR expression was studied and compared with data obtained from 12 voluntary healthy test subjects.

Results Patients with burns: twenty six patients (24 males and 2 females, 30 eyes). Mean age was 43.71 ± 17.8 years. Normal subjects control population: 12 patients 5 females and 7 males, 18 eyes. Mean age: 32 ± 8.24 years. HLA-DR expression increased in all patients after two months. In most patients a strong level of HLA-DR expression remained between 6 and 12 months even when slit lamp examination seemed normal. After 12 months HLA-DR expression levels dropped dramatically in most patients and were not statistically different from the ones measured in test subjects after 18 months.

Conclusion IC is a new useful noninvasive tool for the follow-up of severe alkali ocular burns

■ 2236**The Implication of TLR's in Sjögren's Syndrome and severe Dry eye**

LANGMAN ME (1, 2)

(1) St Louis Hospital / Ophthalmology Department, Saint Jean d'Angély

(2) Jean Bernard University Hospital / Ophthalmology Department, Poitiers

Purpose NKT lymphocytes are nonconventionallymphocytes present in hematopoietic tissues, but also in lymphatic ganglia, digestive tract associated lymphoid tissue and in the dermal tissue. They show at the same time the phenotypical characteristics of the Natural Killer (NK) cells but also of the conventional T lymphocytes. On the other hand, they use a receptor for the invariant antigen, called TCR (T cell receptor), which is characteristic. NKT cells are implied in the physiopathology of several dermatological affections such as psoriasis and sclerodermy. In psoriasis CD1d expressing keratinocytes stimulate NKT lymphocytes which exclusively produce IFN γ . This IFN γ production maintains CD1d expression. We studied the expression of CD1d and of TLR 2, 4 and 9 by the various cell lines components of the ocular surface and tested in confocal microscopy and flow cytometry, the presence of CD1d and of the various TLRs on conjunctival epithelial cells collected by impression cytology, carried out on voluntary test subjects (free from ocular pathologies) or presenting with chronic ocular surface inflammation. We carried out a functional study of CD1d and of TLRs expression in corneal and conjunctival cell lines after various stimulations by cytokines.

■ 2241

Mitochondrial dysfunction and cell death: focus on α-lipoic acid

OSBORNE NN

Nuffield Laboratory of Ophthalmology, Oxford University, Oxford

Purpose Retinal ganglion cell axons are particularly enriched with mitochondria. When mitochondria are drastically impaired, as might occur glaucoma, ganglion cell apoptosis occurs. Thus agents targeted specifically at enhancing ganglion cell mitochondrial energy production and/or scavenge free radicals (generated by dysfunctional mitochondria) might be useful in the treatment of a disease like glaucoma. One possible substance worthy of consideration is possibly α-lipoic acid. It is tolerated when taken orally, diffuses readily into cells, serves as a co-factor to mitochondrial enzymes and is also a very powerful antioxidant.

Methods Rats were injected intraperitoneally with either vehicle or α-lipoic acid (100 mg/kg) once daily for 11 days. On the 3rd day, ischemia was delivered to the retina by raising the intraocular pressure above systolic blood pressure for 45 min. The electroretinogram was measured prior to ischemia and 5 days after reperfusion. Rats were killed 5 or 8 days after reperfusion and the retinas processed for immunohistochemistry and for different mRNA levels by RT-PCR.

Results Ischemia-reperfusion caused a significant reduction of the a- and b-wave amplitudes of the electroretinogram, a decrease in nitric oxide synthase and Thy-1 immunoreactivities, a decrease of retinal ganglion cell-specific mRNAs and an increase in bFGF and CNTF mRNA levels. These effects were blunted by α-lipoic acid.

Conclusion The results show that α-lipoic acid provides protection to the retina and their ganglion cells from ischemia-reperfusion injuries. It is also known that α-lipoic acid blunts any loss of retinal neurons in culture caused by anoxia or light. The combined data therefore suggests that α-lipoic acid should be considered a candidate drug to treat glaucoma patients.

■ 2242

Reducing the risk of AMD: diet vs supplements

KLAVER CCW, VAN LEEUWEN R, DE JONG PTVM

-, Rotterdam

Purpose Antioxidants may interfere with the development of age-related macular degeneration (AMD) by reacting with the free radicals that are produced in the process of light absorption by the retina. In the Age-Related Eye Disease Study (AREDS), supplements containing 5 to 13 times the recommended daily allowance of β-carotene, vitamins C and E, and zinc resulted in a 25% reduction in the 5-year progression from early to late AMD. We sought to investigate whether antioxidants present in a normal diet may play a role in prevention of AMD.

Methods In the population-based Rotterdam Study, dietary intake was assessed in 4765 subjects (aged >= 55 yrs) at baseline using a semi-quantitative food frequency questionnaire. Incident AMD was graded on fundus photographs at three follow-up visits with a total mean follow-up time of 8.0 years. We estimated the risk of AMD associated with the dietary intake of antioxidant nutrients with Cox proportional hazards regression analysis.

Results Incident AMD occurred in 560 participants. When antioxidants were examined separately, only vitamin E and zinc showed a significant inverse association with incident AMD. The hazard ratio (HR) per standard deviation increase of intake for vitamin E was 0.92 (95% confidence interval [CI], 0.84-1.00) and for zinc was 0.91 (95% CI, 0.83-0.98). However, when the combination of antioxidants was examined, an above-median intake of all 4 nutrients that were given in the AREDS Study was associated with a 35% reduced risk (HR 0.65; 95% CI, 0.46-0.92) of AMD. Exclusion of supplement users did not affect the results.

Conclusion Our data suggest that a high intake of antioxidants from a regular diet may already delay the development of AMD, and intake of doses exceeding the recommended daily allowance may not be obligatory.

■ 2243

Lutein and zeaxanthin: genetics and supplementation

VAN KUIJK E (1), LIEW M (2), BEATTY S (3), MELLERIO J (4), MARSHALL J (4), HAMMOND C (5), GILBERT C (6)

(1) *Ophthalmology & visual Sciences, Galveston, TX*(2) *Moorfields Eye Hospital, London*(3) *Ophthalmology, Waterford*(4) *Ophthalmology, London*(5) *West Kent Eye Center, Orpington*(6) *London Sch of Hygiene & Trop Med, London*

Purpose The amount of macular pigment (MP) and its distribution profile are possible risk factors in the pathogenesis of age-related macular degeneration (AMD). MP appears to decrease in AMD and supplementation with lutein and/or zeaxanthin may alter the amount of MP or its distribution profile.

Methods 324 healthy volunteers (mean age was 39 +/- 8.7 years, range 16-50) were recruited as part of a twin eye study. Subjects were asked to take a daily vitamin supplement, "Macuvite" (Springfield), consisting of 18mg lutein (in its free form) and 2.4mg of zeaxanthin for 6 months. Macular pigment optical density (MPOD) was measured in the right eyes at 0, 3 and 6 months of all subjects using heterochromatic flicker photometry (HFP) and a 2-wavelength (488nm & 514nm) fundus autofluorescence (AF) technique, while monitoring compliance.

Results At baseline, MPOD levels exhibited a mean of 0.44 +/- 0.21 (range 0.04 to 1.25) using HFP and 0.4 +/- 0.15 (range 0.03 to 0.85) using AF (at half-degree eccentricity). At 3 months following supplementation, there was no increase in MPOD levels, but at 6 months of supplementation, a marginal increase in MPOD was seen using both methods.

Conclusion High dose MP supplementation resulted in no rise in MPOD at 3 months and a small rise at 6 months, despite significant increases in serum MP levels. This suggests that (a) future supplement studies need to be long-term, (b) the formulation of supplementation may be important and (c) further studies are needed to determine the effect of supplementation on the MP optical density profile.

■ 2244

Can the macular pigment be influenced? The LUNA (Lutein nutrition effects measured by autofluorescence) Study

TRIESCHMANN MBT, PAULEIKHOFF DP

Department of ophthalmology St.Franziskus Hospital, Münster

Purpose Can the Macular pigment (MP) in the eye, consisting of Lutein (L) and Zeaxanthin (Z), discussed as a potential protector against age related macular degeneration (AMD), be increased by supplementation?

Methods 108 eyes of 108 subjects (68 females, 40 male, age 51-87, mean 71.6y.) with and without AMD were investigated. Subjects obtained 12mg L and 1 mg Z (Ocuverte Lutein) each day for 6 months. At baseline and after 3 months L and Z were measured in serum and every six weeks the MP optical density (MPOD) was quantified by analysis of grey scale values after digital subtraction of two autofluorescence (AF) images taken at 488 nm (significant absorption by MP) and 514 nm (nearly no absorption by MP) using a modified Heidelberg Retina Angiograph (HRA). The control group consisted of 28 subjects (16 females, 12 males, age 57-83, mean 71.04).

Results Baseline MPOD at 0.5° (MPOD 0.5°, mean 0.489 D.U.) was positively correlated to the L ($p=0.028$) and Z ($p=0.029$) serum concentration. Following intake of L and Z MPOD 0.5° values increased by mean 0.1 D.U. ($p<0.001$) up to three months (visit 6) after stop of supplementation reaching a mean value of 0.59 D.U. The difference in MPOD 0.5° between visit 6 and baseline was separated into four quartiles (Q): mean MPOD 0.5° change Q1 0.48 to 0.47, Q2 0.53 to 0.57, Q3 0.53 to 0.6, Q4 0.42 to 0.58 D.U. Baseline and increase of serum L- and Z levels were similar in all Qs. Increase of MPOD 0.5° in the control group was not significant (+0.030 D.U., $p=0.15$).

Conclusion MPOD measured by a modified HRA increases following intake of 12 mg L and 1 mg Z. The missing increase of MPOD in one quarter is not caused by malabsorption. Low baseline MPOD show strong or no increase.

■ 2245

Zinc, Drusen and Complement Factor H

LENGYEL I

Division of Pathology, Institute of Ophthalmology, UCL, London

Purpose Despite its therapeutic use to prevent late age-related macular disease (AMD), the cellular effects of zinc in AMD are largely unknown. One of the hallmarks of AMD is the appearance of drusen. Previous studies looking at zinc content of drusen were inconclusive. The purpose of this study was to determine the concentration and distribution of zinc in drusen and Bruch's membrane in AMD donor eyes and relate these observations to Complement Factor H (CFH) accumulation and polymorphism.

Methods Using microprobe synchrotron X-ray fluorescence we measured the total zinc concentration in drusen and Bruch's membrane in human donor eyes. Furthermore, using ZP1 (a lipophilic fluorescence sensor for zinc) and confocal microscopy the internal distribution of zinc within drusen was determined.

Results Our microprobe synchrotron X-ray fluorescence measurement presented evidence that millimolar accumulation of zinc in drusen and Bruch's membrane are strongly associated with AMD in human donor eyes. There were local inhomogeneities of biologically active zinc in internal structures within drusen. Similar internal structures were associated with accumulations of CFH (Hageman et al. 2005, PNAS, 102(20):7227-32), suggesting that zinc and CFH may interact in AMD.

Conclusion Based on our observations, modelling studies and the already available literature we hypothesize that changes in zinc homeostasis at the RPE/choroid interface has a direct effect on CFH. Zinc may inhibit and precipitate CFH, especially the Y402H mutant, and together they might be involved in the development and/or progression of AMD, implying a very complex role for zinc in the pathogenesis of AMD.

■ 2251**Role of Vascular Endothelial Growth Factor and Placental Growth Factor in Glaucoma and Scar Formation after Glaucoma Filtration Surgery**

LIZ (1, 2), VAN DE VEL (1, 2), ZEYEN T (3), CARMELIET P (2),
STALMANS I (3, 1, 2)

(1) Laboratory of Ophthalmology, Catholic University Leuven, Leuven
(2) Center for Transgene Technology and Gene Transfer, Catholic University Leuven, Leuven
(3) Dept of Ophthalmology, University Hospital Leuven, Leuven

Purpose Filtering surgery is the most efficient treatment for glaucoma. However, in up to 30%, trabeculectomy fails due to excessive postoperative scarring. This study was designed to elucidate the role of the vascular endothelial growth factor (VEGF) and placental growth factor (PIGF) in fibrosis after glaucoma filtration surgery.

Methods First, aqueous humor and plasma samples were collected from glaucoma and non-glaucomatous (cataract) patients and the concentration of VEGF and PIGF were measured by ELISA. Secondly, the expression of the VEGF receptors Flt-1 and KDR was determined in cultured human Tenon fibroblasts by quantitative real-time RT-PCR and Western blotting. Finally, the effect of VEGF on these Tenon fibroblasts was determined using a proliferation assay.

Results The mean concentration of VEGF and PIGF in aqueous humor of glaucoma patients was significantly higher than that in patients with cataract ($P < 0.05$). No differences, however, were found in the plasma from these patients. The expression of Flt-1 and KDR was detected successfully at the RNA as well as the protein level with quantitative real-time RT-PCR and Western blotting in cultured human Tenon's capsule fibroblasts. Furthermore, cell proliferation test showed that VEGF was able to stimulate the growth of these fibroblasts.

Conclusion VEGF and PIGF are increased in the aqueous of glaucoma patients and may play a role in scarring after filtering surgery. Therefore, a therapy with anti-VEGF or anti-PIGF might open new perspectives for more successful filtering surgery, and thus considerably improve the visual prognosis of glaucoma patients.

■ 2252**Colour Doppler Imaging as a diagnostic tool in Normal Tension Glaucoma**

REMKY A, KALIP M, AREND O, WEBER A, PLANGEN
Augenklinik, Technical University, Aachen

Purpose To evaluate sensitivity and specificity of colour Doppler imaging parameters of retrobulbar vessels in NTG using Receiver Operating Curve (ROC) analysis.

Methods 78 patients with NTG (mean age 60 ± 12 years) and 35 controls (mean age 59 ± 9 years) were included in a retrospective analysis. In the ophthalmic artery (OA), central retinal artery (CRA) and nasal and temporal short posterior ciliary arteries (PCA) flow velocities (peak systolic velocity PSV and end-diastolic velocity EDV) were measured by means of colour Doppler imaging (Siemens Sonoline Sienna). Using Receiver Operating Characteristic (ROC) curves sensitivity was determined at 90% specificity.

Results Intraocular pressure and blood pressure values were not different between patients and controls. Patients with NTG showed significantly decreased PSV ($p=0.001$) and EDV ($p=0.009$) of the CRA and significantly increased RI of the OA ($p=0.047$) compared to controls. The sensitivity values at 90% specificity were determined: PSV of the CRA: 24%, EDV of the CRA: 32%, EDV of the nasal PCA: 27%, EDV of the temporal PCA: 31 %. The PSV and EDV of the OA and the PSV of the nasal and temporal PCA showed sensitivity levels below 15% at 90% specificity.

Conclusion Sensitivity to detect NTG patients with colour Doppler imaging was low for all parameters. The EDV of the CRA was the best parameter with 32% sensitivity at 90% specificity. Assessing retrobulbar hemodynamics is of minor value to discriminate NTG patients from controls. The diagnostic power of colour Doppler measurements for progression needs to be determined in prospective longitudinal studies

■ 2253**Ocular pulse amplitude and color doppler imaging in glaucoma patients versus normal subjects**

STALMANS I (1), SIESKY B (2), VAN BELLINGHEN V (1), ZEYEN T (1),
HARRIS A (2)

(1) Dept of Ophthalmology, University Hospital Leuven, Leuven
(2) Dept of Ophthalmology, Indiana University School of Medicine, Indianapolis, IN

Purpose 1) To evaluate intra- and inter-observer reproducibility of retrobulbar Color Doppler Imaging (CDI). 2) To compare retrobulbar flow velocities as well as Ocular Pulse Amplitude (OPA) between healthy subjects and patients with normal tension or high tension glaucoma. 3) To study the correlation between OPA and CDI parameters.

Methods CDI and OPA measurements were performed twice with one month interval in patients with normal tension (n=30) or high tension glaucoma (n=18), and healthy individuals (n=22). The CDI measurements were performed on the central retinal artery, short posterior ciliary arteries and ophthalmic artery, and analysed by the observer and a masked reading center. Differences were calculated by a student T-test and correlations with Spearman analysis.

Results 1) The CDI measurements were comparable between the two study visits. Moreover, analysis of the CDI pictures by the masked reading center resulted in values that were comparable to those obtained by the observer. 2) OPA as well as CDI parameters were significantly reduced in patients with primary open angle or normal tension glaucoma, as compared to non-glaucomatous individuals ($p=0.000001-0.05$). 3) A correlation was observed between the OPA and the systole/diastole ratio as well as the resistive index in the healthy individuals in all measured blood vessels ($P=0.009-0.05$). This correlation was not obvious in the glaucomatous patients.

Conclusion Retrobulbar CDI is reproducible over time and between reading centers. OPA and flow velocity parameters are significantly reduced in normal tension as well as high tension glaucoma patients. OPA and CDI parameters strongly correlate in healthy individuals, but not in glaucoma patients.

■ 2254**Clinical evaluation of Applanation resonance tonometry – a comparison with Goldmann applanation tonometry**

HALLBERG P (1), EKLUND A (1), BACKLUND T (1), LINDEN C (2)

(1) Department of Biomedical Engineering and Informatics, University Hospital, Umeå
(2) Department of Clinical Science, Ophthalmology Umeå University, Umeå

Purpose The purpose of this study was to evaluate the precision of the new Applanation Resonance Tonometry (ART) in a clinical study designed in accordance with the International Standard Organisation's (ISO) requirements.

Methods This was a prospective, randomised, single-centre study, where healthy volunteers and patients participated. A total of 153 eyes were divided into three groups with respect to their intraocular pressure (IOP) at screening: < 16 mmHg, 16 - 23 mmHg, and > 23 mmHg. IOP was measured with Goldmann applanation tonometry (GAT) as reference method and by ART in both a biomicroscope (ARTBiom) and a handheld (ARTHand) set-up with a 10-min. pause between methods. The mean of six readings were regarded as one measurement value.

Results Mean age of the subjects was 59 yr. (range 20 to 87 yr). GAT showed a mean IOP of 20.0 mmHg (range 8.5 to 43.5 mmHg, n=153). The precision was 2.07 mmHg for ARTBiom and 2.50 mmg for ARTHand, with a significant dependency for age as compared with GAT. Measurement order produced a decreasing IOP with a mean of 2.3 mmHg between the first and last method.

Conclusion The precision obtained in both ARTBiom and ARTHand was within the limits set by the ISO standards for tonometers. The standardised procedure and the stability of the biomicroscope set-up resulted in a slightly better precision as compared with the handheld set-up. Despite a 10-min. pause between measurements, the order was a significant factor, possibly because the patients were more apprehensive at the first measurement.

■ 2255**Correlation Between Ocular Response Analyzer, Dynamic Contour Tonometry, and Goldmann Applanation Tonometry**

RENIER C, ZEYEN T, STALMANS I

Dept. of Ophthalmology, Leuven

Purpose Goldmann applanation tonometry (GAT) is gold standard for intraocular pressure (IOP) measurement. However, biophysical properties of the cornea affect the accuracy of the measurements. New tonometers such as ocular response analyzer (ORA) and Pascal dynamic contour tonometry (DCT) are thought not to be influenced by the central corneal thickness (CCT). The aim of our study is to compare the IOP measurements obtained by ORA, DCT, and GAT, and to assess the correlation between CCT and the IOP measured by the three instruments.

Methods In 41 patients, IOP was measured in a single eye with GAT, and 30 minutes later with ORA and DCT by an independent observer. CCT was measured with an ultrasound pachymeter (Pachmate). We compared the mean IOP measurements between the three instruments with a multivariate normal model. The correlation between IOP measurements and CCT was assessed with Spearman correlation coefficients. Bland & Altman plots were used to evaluate the agreement between the tonometry methods.

Results The mean IOP measured with ORA and DCT was comparable ($p=0.69$). There was a significant difference between the mean IOP measured with ORA and GAT ($p<0.0001$), as well as between the mean IOP measured with DCT and GAT ($p<0.0001$). There was no correlation between the IOP measurements and CCT for the three instruments ($p>0.7$). Bland & Altman graphs showed disagreement between the measurements taken with the three tonometers.

Conclusion Our study showed disagreement between IOP measured by ORA, DCT and GAT. No correlation was found between CCT and IOP values obtained by the three instruments.

■ 2256 / 417**Inducible NOS synthase in the human trabecular meshwork (TM) from patients with primary open-angle glaucoma (POAG)**

FERNANDEZ DURANGO R (1), GARCIA FEIJOO J (2), CASTILLO A (2),

MARTINEZ DE LA CASA JM (2), GARCIA-BUENO B (3),

FERNANDEZ-CRUZA A (4), LEZA JC (3)

(1) Unidad de Investigación, Hospital Clínico San Carlos., Madrid

(2) Ophthalmology, Hospital Clínico San Carlos., Madrid

(3) Pharmacology, Complutense University, Madrid

(4) Internal Medicine, Hospital Clínico San Carlos, Madrid

Purpose Visual field damage and IOP in patients with POAG has been correlated with inflammatory parameters in TM. Constitutive and inducible nitric oxide synthase (NOS) activities and expression were analyzed in TM.

Methods TM specimens were obtained at filtration surgery from 60 glaucoma patients. Constitutive and inducible (iNOS) activities were measured by the conversion of L-[14C]-arginine to L-[14C]-citrulline method. NOS expression was detected by real-time RT-PCR. Levels of malondialdehyde (MDA), were evaluated in the aqueous humor (AH) of control (cataract n=16) and POAG patients (n=17). Patients underwent a Octopus G1 visual field examination and tonometry before surgery.

Results Constitutive, Ca²⁺-dependent activity of the TM from the patients with MD between 2 and 12 dB was similar (120 ± 60 pmol.min⁻¹ mg prot⁻¹) but not detectable in the patients with MD>12 dB. No differences were found on the expression of the main constitutive isoform in TM (eNOS). iNOS mRNA and activity were only detected in the TM of patients with MD>12 dB (396 ± 56 pmol.min⁻¹ mg prot⁻¹). MDA levels correlated with iNOS activity (control: 0.05 ± 0.003 nmol/ml; POAG: 0.07 ± 0.001 nmol/ml; $p<0.05$). No other correlations were found.

Conclusion The expression and activity of the high-output source of NO are increased in the TM of POAG patients with severe visual field damage, an effect that is paralleled with MDA accumulation in AH. Constitutive NOS activity is reduced as the severity of POAG increases. These results suggest that excessive synthesis of NO produced by iNOS may damage TM cells and increases the severity of the loss of the visual field in these patients.

■ 2257 / 437**Intraocular pressure measurement using near infrared spectroscopy**

KAMPPETER BA (1), WITZENMANN T (2), BACKHAUS J (2), JONAS JB (1)

(1) Department of Ophthalmology, Faculty of Clinical Medicine Mannheim, Mannheim

(2) University of applied sciences, Mannheim

Purpose Despite advanced modern technology measurement of intraocular pressure is still a challenge. All systems in use depend on an indirect principle of measurement, influenced by tissue parameters. A new non invasive system to measure the intraocular pressure based on near infrared spectroscopy might overcome these limitations, by offering a real direct, non contact and non invasive intraocular pressure reading. It is based on the specific molecule absorption of near infrared radiation which is characteristically for individual molecule configurations. This principle is now being used in the human eye by measuring water molecule oscillation in the anterior chamber to assess the intraocular pressure transcorneally.

Methods 226 eyes from 113 patients were included in this study. Due to the impairment of existing pressure measuring devices, three different measuring techniques as reference methods for average calculation were used. Following standard Goldmann applanation tonometry, corneal thickness was evaluated and Goldmann readings were adjusted according to Shah. In addition, Dynamic contour tonometry (Pascal Tonometer; SMT Swiss Mircotechnology AG, Zurich, Switzerland) was performed.

Results Intraocular pressure readings ranged between 8 and 52mmHg (mean: 17.2 ± 5.8 mmHg). Mean discrepancy between near infrared spectroscopy's pressure values and mean of standard techniques was 4.43 ± 3.04 mmHg (Coefficient of correlation: $r^2=0.95$, RMSECV=2.2).

Conclusion Near infrared spectroscopy showed to be applicable in a real life environment for intraocular pressure measurement. Considering the lack of an objective intraocular pressure measuring device, results of this study suggest a high correlation between near infrared spectroscopy and standard devices.

■ 2261

Tips and Tricks in Paediatric Retinal Electrophysiology

HOLDER GE

Moorfields Eye Hospital, London

Purpose To describe the techniques and applications of ERG in a paediatric population.

Methods The basics of ERG recording will be described with the use of corneal recording electrodes in older children and surface (eyelid) electrodes in younger children and babies. VEP recording is sometimes a necessary adjunct.

Results Data will be presented illustrating the diagnostic use of electrophysiology in a variety of disorders.

Conclusion The objective data provided by electrophysiological examination has a privileged position in the assessment of a child, particularly when they are unable to give an accurate history or description of their symptoms

■ 2262

Molecular Genetic Analysis of Early Onset Severe Retinal Dystrophies EOSRD

PREISING MN

Dept. of Paed. Ophthalmology, Strabismology and Ophthalmogenetics, Klinikum, University of Regensburg, Regensburg

Purpose Molecular genetic screening in early onset severe retinal dystrophies (EOSRD) including Leber's Congenital Amaurosis (LCA) and autosomal recessive or unspecific Retinitis pigmentosa with early onset (RP) identified eight candidate genes (GUCY2D, RPE65, AIPL1, CRB1, RPGRIP, LRAT, TULP1, CRX) during the past nine years. As time went by those involved in untackling EOSRD recognized that they had found the tip of a growing iceberg. New developments like mutation specific microarray analysis, resequencing microarrays and automated conformation analysis and sequencing will help identify the patients of interest for further gene therapy trials. Nevertheless, further development of approved methods like linkage analysis using SNP microarrays will be necessary to face the challenge of multifactorial disorders.

Conclusion This presentation summarizes the research in EOSRD and conclusions drawn from it over the past years.

■ 2263

Dissecting the Clinical Phenotype of EOSRD

LORENZ B

Dept. of Paediatric Ophthalmology and Ophthalmogenetics, Klinikum, University of Regensburg, Regensburg

Purpose To describe the possibilities and limitations of longitudinal phenotyping in EOSRD as current achievements with molecular genetic methods and the perspective of specific therapeutic options in childhood-onset retinal dystrophies, and in particular in EOSRD.

Methods A set of more recent diagnostic techniques like fundus autofluorescence (AF), optical coherence tomography (OCT), 2 colour threshold perimetry (2CTP), and spectral sensitivity (SS) measurements in addition to advanced electrodiagnostics are used to describe function and morphology not only in patients but also in parents who according to the genotype may show clinically asymptomatic pathologies.

Results Due to limited capacities and compliance in young children, high-resolution testing is still challenging at that age. Non-invasive objective methods with short testing times are ideal. Subjective methods are more difficult but their efficiency can be improved by adapting them to age. Quantifying function in advanced stages of the disease also requires the development of new tests. This paper summarizes actual achievements combining advanced imaging methods with electrophysiological and psychophysical testing. Special emphasis is given on the need for age-adapted normative values and feasibility and reliability of the various methods in dependence on age and disease stage.

Conclusion Evolving age-adapted methods of high-resolution *in vivo* phenotyping allow to dissect the clinical phenotype of EOSRD for precise genotype-phenotype correlation, and as a prerequisite for future therapeutic interventional trials.

■ 2311

PEDF in Development, Diseases, and Therapeutics

TOMBRAN-TINK J, BARNSTABLE CJ

Department of Ophthalmology, Yale University School of Medicine, New Haven, CT

Purpose Neural repair strategies using endogenous molecules are of significant clinical importance because they focus on tools harnessed by the body's own neuroprotective mechanisms to maintain function and survival of neurons that are bombarded by a range of toxic fluctuations in vivo. Pigment Epithelial Derived Factor (PEDF), a 50 kDa pleiotrophic glycoprotein, is among the list of such molecules with pronounced survival effects in experimental models that mimic various aspects of neurodegenerative diseases. The protein is synthesized and secreted early in development by several cell types and is present in many fluid compartments of the body including the CSF, vitreous and aqueous humor, and in plasma. The neuroprotective efficacy of this protein against glutamate excitotoxicity has been shown in cerebellar granule cells, hippocampal neurons, and spinal cord motor neurons. Similar effects are observed in the retina where light damage, increased hydrogen peroxide levels, and retinal detachment from the RPE layer result in the degeneration of photoreceptor cells and Muller glia cell dysfunction. Two major signaling pathways associated with PEDF's actions are NF κ B and ERK1/2 MAP kinase pathways. The therapeutic potential of PEDF is evident in intraocular injections of soluble full-length PEDF protein or adenoviral mediated transfer of the gene. Such PEDF preparations increase survival of photoreceptors in the rd1 and rds genetic mouse models of photoreceptor degenerations and prevent apoptosis of retinal ganglion cell during ischemic injury. We have recently identified small PEDF peptide mimetics that not only protect neurons but that can also be delivered in nanoparticle formulations with extended neuroprotective actions in vivo. Supported by grants from the NIH and the David Woods Kemper Memorial Foundation

■ 2312

Signalling pathways in photoreceptor and ganglion cell responses to neurotrophic factors

HICKS D, SIFFROI-FERNANDEZ S, BUGRA K, SWAROOP A, BONNET D

Laboratoire de Neurobiologie des Rythmes, CNRS UMR 7168/LC2, Strasbourg

Purpose To characterise the signalling cascades activated in defined retinal cell populations by candidate neurotrophic and soluble factors.

Methods Primary cell cultures of different retinal cell populations – rod and cone photoreceptors (PR), Müller glial cells (RMG) or mixed neuronal cultures containing retinal ganglion cells (RGC) – were prepared from adult pig or newborn rat retinas. Cultures were treated with candidate neurotrophic factors (NTFs) – BDNF, different members of the FGF family, PEDF, retinoic acid – and depending on the target population, different parameters were measured – cell survival, neurite extension, proliferation, protein expression. Corresponding NTF receptors, intracellular messengers, and candidate transcription factors, were analysed by immuno-histochemistry, RT-PCR, immuno-precipitation and immuno-blotting.

Results Cell biological effects could be correlated with presence and activation of distinct pathways depending on the particular population in question. PR express high levels of FGFR-4 and displayed increased survival in the presence of FGF-19; RMG and RGC express high levels of FGFR-3 and showed increased proliferation or survival respectively following addition of FGF-9; PEDF and BDNF stimulated survival and neuritogenesis of RGC; retinoic acid and FGF-19 up-regulated expression of the rod PR-specific transcription factor NRL.

Conclusion Use of well-defined in vitro models allows in-depth examination of NTF effects and signalling.

■ 2313

CNTF Cascades in Retinal Development and Disease

BARNSTABLE CJ, TOMBRAN-TINK J

Yale University School of Medicine, New Haven, CT

Purpose Ciliary neurotrophic factor (CNTF) is a member of the IL-6 family of cytokines and activates both STAT3 and MAPK intracellular signalling pathways. Treatment of embryonic and early postnatal mouse retinas with CNTF blocks the expression of opsin. Using retinal explant cultures and cDNA microarrays, we have generated a gene expression profile comparison between CNTF treated and untreated retinas. 7 days after CNTF treatment, a sample of 10 rod photoreceptor genes were all decreased at least 2 fold by CNTF ($\log_2 > -1$), indicating that this factor has a general suppressive effect on rod photoreceptor development. CNTF also affects expression of other retinal genes and may regulate the development of multiple retinal cell types. By using adenoviruses containing wild type or dominant negative forms of STAT3, as well as pharmacological inhibitors of MAPK activation, we have shown that inhibition of rod photoreceptor development depends upon the activation of the STAT3 pathway and not the MAPK pathway. On the other hand, CNTF mediated activation of the MAPK pathway plays a crucial role in the development of Müller glial and bipolar cells. A major action of CNTF may be to prevent or delay the exit of retinal progenitor cells from their proliferative state into their terminal differentiation pathway. Like a number of other factors, CNTF is neuroprotective in a wide range of experimental models of retinal degeneration. CNTF prevents ganglion cell death induced by several insults. Manipulation of STAT3 expression in ganglion cells shows that this protective effect is dependent upon this protein. A common target of several neuroprotective factors is the Müller glial cell. Such comparisons may allow us to pick out key neuroprotective pathways that will allow us to develop rational combinatorial therapies so as to optimally exploit the functions of each factor. Supported by grants from the NIH and the David Woods Kemper Memorial Foundation.

■ 2314

Role of GDNF in the differentiation of photoreceptors in 3D-retinal spheres of the chick embryo

LAYER PG (1), VOLPERT K (1), ROTHERMEL A (2)

(1) Biology, Darmstadt

(2) CBB University Leipzig, Leipzig

Purpose To investigate roles of glial cell line-derived neurotrophic factor (GDNF) and one of its receptors GFR α 4 on proliferation, differentiation and apoptosis in a three-dimensional histotypic in vitro model of the chick embryonic retina.

Methods Retinal spheres were reaggregated from 6-day old chicken retina under permanent rotation. In a first series, they were cultured in presence of 50 ng/ml GDNF under reduced serum conditions. Since an ISH study had established the expression of GFR α 4 in the in vivo retina and in spheres, in a second series, spheres were transfected with GFR α 4 siRNA at culture day 2. The temporal differentiation of rods, cones and other cell types was analysed by immunohistochemistry, RT-PCR and ISH.

Results GDNF supplementation led to an earlier differentiation of rods and an increase of their numbers, while cones and other retinal cell types were not much affected. In non-treated older cultures, rods but not cones underwent apoptosis; rod apoptosis was strongly inhibited by GDNF. An efficient knock-down of GFR α 4 receptor reduced the size of spheres and inhibited cell proliferation, thereby decreasing blue cones and Pax6-positive cells.

Conclusion GDNF supplementation strongly supports rod differentiation and their maintenance in vitro. This role seems not to be exerted via the GFR α 4 receptor. The complexity of interactions of GDNF with its various receptors and other growth factors need much further investigations and can be appropriately determined in retinal spheres; first results of GDNF plus PEDF co-actions will be reported. References: Rothermel A and Layer PG (2003). IOVS 44, 2221-2228; Rothermel A et al. (2004). Gene Expr Patterns 4, 59-63; Rothermel A et al. (2006). IOVS 47, 2716-2725.

■ 2321

Acute anterior (kerato)uveitis

DE SCHRYVER I, KESTELYN P

Department of Ophthalmology, Ghent University Hospital, Ghent

Purpose To describe the presentation, differential diagnosis and therapeutic approach in acute anterior uveitis and keratouveitis.

Methods A case presentation will be used to illustrate different clinical presentations of acute anterior uveitis and keratouveitis.

Results Although anterior (kerato) uveitis can be idiopathic, many underlying infectious and inflammatory conditions are identified. Many diseases that can cause a panuveitis, may start as an anterior uveitis. Both differential diagnosis and therapy will be discussed.

Conclusion Early diagnosis and prompt treatment of acute keratouveitis is needed since associated complications such as glaucoma may result in severe visual loss.

■ 2322

Long term survival in sarcoid uveitis

MISSOTTEN T (1), VAN DAELE PL (2), VAN LAAR JAM (2), VANDER LOOS T (3)

VAN HAGEN PM (2), BAARSMA GS (1)

(1) Uveitis dept Rotterdam Eye Hospital, Rotterdam

(2) Immunology dept Erasmus University, Rotterdam

(3) Internal medicine dept Rotterdam Eye Hospital, Rotterdam

Purpose Sarcoidosis is a granulomatous multisystem disorder of unknown cause. In over 20% of patients, anterior uveitis (AU), intermediate uveitis (IU) or a panuveitis (PAN) with vasculitis and chorioretinal infiltrates is present. Although regarded a mild uveitis entity, in comparison to other non-infectious uveitic disorders as Behcet's uveitis or sympathetic ophthalmia, no precise data are available on longterm outcome.

Methods The uveitic data of 154 sarcoid patients (288 eyes) who visited the Rotterdam Eye Hospital Uveitis department between 1-1-2005 and 31-12-2005, and agreed to have their data used for this study, was analysed retrospectively.

Results Onset of sarcoid uveitis (43.6 ± 14.6 y, range 10-83 y) was not equally distributed between men and women: In caucasian females, a late onset sarcoid uveitis was found between ages 60-80y. Follow-up (7.6 ± 6.8 y, range 3mth-28.8y) accumulated to 1171 patient years. Anterior segment (AU) activity, vitritis and vasculitis was most prominent in the first 5 years of disease. In 20% of patients, activity persisted after 5 years post onset, and became only quiescent at about 20y post onset. Disease subdivision at 10 y post onset was AU 15%, IU 55%, PAN 30%. Cumulative incidence of CME at the 20y mark was 42.4%. BCVA at 1, 5, 10 and 20 years post onset was 0.7 ± 0.3 snellen. Cumulative legal blindness incidence at 20 years post onset was calculated to be 14.3% per eye, or 2% for bilateral blindness.

Conclusion This study provides clinicians and patients information on longterm prognosis. With current non-biological therapy, visual acuity in sarcoid eyes at the 20 years post onset of disease mark is good (0.7 ± 0.3 snellen). Nevertheless, patients have a 2% chance of bilateral legal blindness.

■ 2323

Optimal pulmonary investigations in patients with uveitis of suspected sarcoidosis origin

EBRAERTH (1), MICHEL O (2), WILLERMAIN F (3, 4), CASPERS L (5, 4)

(1) AZ VUB Ophthalmology, Brussels

(2) CHU St-Pierre Pneumology, Brussels

(3) CHU St-Pierre Ophthalmology, Brussels

(4) Brugmann Ophthalmology, Brussels

(5) CHU St-Pierre Ophthalmology, Brussels

Purpose To investigate the pulmonary involvement in patients with uveitis of unknown origin suspected of sarcoidosis, by broncho-alveolar lavage (BAL) and chest imaging (X-ray or CT scan).

Methods We have prospectively evaluated 112 patients with suspected sarcoidosis uveitis. There were 34 females and 78 males; 19 were smokers. All patients had a complete ophthalmological examination, a broncho-alveolar lavage, (BAL), a chest X-ray and/or thoracic CTscan.

Results Among the population, a normal chest X-ray was observed in 91 (81.2%) while 21 were suggestive of lung sarcoidosis. In our group of patients stage 1, stage 2 and stage 3 was observed in 10 (8.9%), 6 (5.4%) and 5 (4.5%) patients, respectively. The cell population of the BAL was normal in 53 (47.3%) patients; 59 (52.7%) had a significant increase of the percentage of alveolar lymphocytes (>15%). An increased CD4/CD8 ratio (>3.5) in BAL was found in 26 patients (23.2%), highly suggesting sarcoidosis. Interestingly, 17 among 26 (65.4%) patients with an increased CD4/CD8 had normal thoracic imaging.

Conclusion In patients with granulomatous uveitis suggesting of sarcoidosis, CD4/CD8 ratio in BAL may be helpful in detecting an ocular and pulmonary sarcoidosis, even when chest imaging is normal.

■ 2324

Infliximab for the treatment of chronic uveitis

SEN HN (1, 2), LEVY-CLARKE G (1), NUSSENBLATT RB (3)

(1) National Eye Institute, Bethesda, MD

(2) George Washington University, Washington, DC

(3) National Eye Institute, Bethesda, MD

Purpose To evaluate the effectiveness of infliximab infusions in patients with chronic recalcitrant uveitis

Methods Clinical charts of 10 patients with chronic recurrent uveitis seen at NEI and treated with infliximab infusions between 2003-2006 were retrospectively reviewed. Infliximab was started at 3mg/kg if uveitis was quiet and at 5mg/kg if it was active. Infliximab was administered at baseline, 2, 6wks and every 4 wks thereafter

Results There were 5 patients with Behcet's, 1 sarcoidosis, 1 VKH, 2 JRA-associated uveitis and 1 idiopathic retinal vasculitis. 3 were female, 7 were male; mean age was 37.4, mean duration of uveitis was 9.6 yrs; mean follow-up on infliximab was 22.1 months (median:24.5). Infliximab was started when uveitis was active in 5/10. Mean time to quiescence was 3 weeks. Prior to initiation of infliximab mean number of systemic immunosuppressives was 2.5, it was 1.5 after infliximab. Mean concomitant immunosuppressive grading decreased from 8.6 points to 4.0 after infliximab therapy ($p<0.0001$). Mean recurrence rate decreased from 3/yr to 0.2/yr while on infliximab ($p<0.0001$); 8/10 patients achieved complete quiescence with no flare-ups. 6/7 patients with CME showed improvement. All patients either maintained or gained 10 ETDRS letters of acuity during treatment except for one. Side effects attributable to infliximab included a skin abscess, oral candidiasis and non-TB mycobacterial pneumonia in another patient.

Conclusion Infliximab may be considered as a viable alternative in patients with chronic recalcitrant uveitis who have developed intolerance to conventional immunosuppressives; however patients should be monitored closely for potentially serious side effects.

■ 2325

Intravitreal Bevacizumab (AvastinTM) as a potent treatment for refractory macular oedema in patients with uveitis

MACKENSEN F (1), HEINZ C (2), HEILIGENHALS A (2), BECKER MD (1)

(1) *Interdisciplinary Uveitis Center, Heidelberg*(2) *Uveitiszentrum, St. Franziskus Hospital, Münster*

Purpose Bevacizumab is a monoclonal antibody to vascular endothelial growth factor, which has been developed for the treatment of colorectal adenocarcinoma. Recently it has been successfully used for the treatment of age related macular degeneration with choroidal neovascularizations (CNV) and in some cases of central vein occlusion. We treated patients with sight threatening uveitic macular edema which had been refractory to previous medications.

Methods We treated patients with inactive uveitis but persisting macular edema that had been treated unsuccessfully with at least 2 different drugs. Each patient was examined previously to the injection including optical coherence tomography (OCT) measurements. Injection was performed in a sterile environment; 2.5 mg of Bevacizumab was injected intravitreally. Follow-up exams were performed on week 1, 2, 4, 6 and then every 2-4 weeks. Improvement was judged by gain in visual acuity (VA) and thickness reduction in OCT.

Results 6 eyes of 6 Patients were injected. Longest follow-up was 14 weeks (n=1), shortest 1 week (n=6). All patients but one showed reduction in OCT with a median of 63 µm (Range 6-305 µm) already 1 week after injection. Concurrent improvement in VA was seen in 5 of 6 patients, in 3 patients > 2 lines, 2 patients improved only 1 line and one stayed the same but had macular scarring. Of the 5 responding patients 3 remained stable after 14, 8 and 6 weeks and 2 required reinjections after 4 and 8 weeks.

Conclusion Intravitreal Bevacizumab seems to be a possible treatment in the management of refractory inflammatory macular edema. This is only a small case series and short follow-up. Longer follow-up will show if the effect is long-lasting or transient.

■ 2326

Use of Ocular Hypotensive Prostaglandin Analogues in Uveitis: Potential Effects on Anterior Uveitis and Cystoid Macular Oedema

GURBAXANI A (1), LIGHTMAN S (1), CHANG JH (2), MCCLUSKEY P (2)

(1) *Moorfields Eye Hospital, London*(2) *Department of Ophthalmology, St Vincent's Hospital, Sydney*

Purpose To report the clinical experience in the use of prostaglandin (PG) analogues in the treatment of raised intraocular pressure (IOP) in uveitis and their potential effect on the development of anterior uveitis and CME in uveitic eyes.

Methods A retrospective case-controlled study of 163 eyes of 84 patients with uveitis and raised IOP treated with a PG analogue were studied. Relevant clinical data was abstracted from suitably identified consecutive patients at our tertiary referral uveitis clinics over a 3-month period. Control eyes were selected as those uveitic eyes of the same patients, treated with topical IOP-lowering agent(s) other than a PG analogue. Pre-treatment IOP was compared to the mean IOP during PG analogue treatment. The development of anterior uveitis and CME during treatment was between the two groups.

Results Significant IOP reductions were observed during PG analogue treatment. No significant difference in the frequency of anterior uveitis development in the control and study groups($P>0.05$, Fischer's exact test). None of the 69 uveitic eyes without a previous history of CME developed this complication during PG treatment. There was no association between the development of CME and treatment with a PG analogue ($P>0.05$, Fischer's exact test). PG treatment was generally well tolerated.

Conclusion To the authors' knowledge, this is the first study to report the use of PG analogues in the treatment of raised IOP in uveitis. It shows that PG analogues can be effective in the treatment of raised IOP in uveitis without any appreciably increased risk for the development of anterior uveitis or CME, within the limitations of its sample size and study design.

■ 2331

Alpha 11 integrin in the human cornea – importance in development and disease

BYSTRÖM B (1, 2), GULLBERG D (3), PEDROSA-DOMELLÖFF (1, 2)

(1) Dept of Clinical Sciences, Ophthalmology, Umeå

(2) Dept of Integrative Medical Biology, Section for Anatomy, Umeå

(3) Dept of Biomedicine, Section for Physiology, Bergen

Purpose To examine the distribution of integrin alpha 11 chain during fetal development, in normal and diseased adult human corneas.

Methods Six fetal corneas, 10-20 weeks of gestation (wg), and 18 adult corneas (3 normal, 7 keratoconus, 5 bullous keratopathy (BKP), 2 Fuchs' dystrophy and one with a scar after previous deep lamellar keratoplasty (DLKP)) were processed for immunohistochemistry with a specific antibody against integrin alpha 11 chain.

Results At 10-17 wg alpha 11 integrin chain was present in the corneal stroma, especially in the anterior portion. At 20 wg the staining was weak. Normal adult corneas were only weakly stained. The anterior portion of the stroma was strongly stained in the keratoconus corneas. The BKP corneas showed variable and generally weak staining. The Fuchs' dystrophy corneas and the DLKP cornea were unstained.

Conclusion Alpha 11 integrin is a receptor for interstitial collagens. The presence of alpha 11 integrin chain during early corneal development and the novel expression pattern in scarred keratoconus corneas indicates that alpha 11 integrin chain likely plays an important role in collagen deposition during corneal development and in disease with a scarring component. Further studies are underway in an animal model of corneal scarring.

■ 2332

Expression of Neoepitope BKS-1 and distribution of collagen fibrils diameter in normal and keratoconus cornea

AKHTAR S (1), BRON AJ (2), KERR BC (3), CATERSON B (3), HUGHES C (3), MEEK KM (1)

(1) School of Optometry and Vision Sciences, Cardiff University, Cardiff

(2) Nuffield Laboratory of Ophthalmology, Oxford University, Oxford

(3) School of Biosciences, Cardiff University, Cardiff

Purpose Keratan sulphate (KS) is the predominant glycosaminoglycan (GAG) of the corneal stroma. In this study we investigated the relationship between collagen fibril diameters and the precise distribution of KS-chains in normal and keratoconus corneas.

Methods Four normal and four severe keratoconus corneas were used for the study. The distribution of KS-GAGs was investigated by using a MAb (BKS-1) that specifically recognises a keratanase-generated neoepitope [N-acetyl-glucosamine-6-sulphate (GlcNAc-6-S)], therefore allowing precise quantitative determination of the number of KS chains. It was used with western blot and immunogold electron microscopy to quantify the labelling of KS-GAG chains. Collagen fibril diameters were measured using the AnalySYS soft imaging system.

Results Western blot studies showed large numbers of smaller molecular weight KS molecules (25– 40kDa) present in keratoconus cornea than in normal cornea. Immunogold labelling of KS-GAG in keratoconus cornea was significantly ($p<0.01$) less compared to the normal cornea. The collagen fibril diameters was also significantly smaller in keratoconus cornea compared to normal cornea ($p<0.001$).

Conclusion The results suggest that there are more KS GAGs in the normal cornea than in keratoconus cornea. Keratan sulphate plays an important role in stromal collagen fibril assembly and a dysregulation of KS synthesis in keratoconus could lead to changes in collagen fibril diameters.

■ 2333

Glycogen synthase kinase (GSK-3) inhibitors prolong the life of human lens epithelial cells

KARLSSON J-O (1), PETERSEN A (1), ZETTERBERG M (2)

(1) Institute of Anatomy and Cell Biology, University of Göteborg, Göteborg

(2) Department of Ophthalmology, University of Göteborg, Göteborg

Purpose GSK-3 is an evolutionary conserved S/T kinase which regulates cell fate determination in diverse organisms. In this study we wanted to elucidate the role of GSK-3 for cell survival and apoptosis in the human lens, with special emphasis on mitochondrial function.

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 Kenpaualone (2 μ M) for times up to two months. Mitochondrial potential was evaluated with JC-1 and Rhodamine 123. Peroxides: DCFH-DA Superoxide: Hydroethidine (HET)GSH (Glutathione): Monochlorobimane (MCB) Proteolytic activity (caspases, calpain, metalloproteases and the proteasome): Fluorogenic peptides substrates

Results Long-term inhibition of GSK-3 prolonged the life of cultured HLEC. The mitochondrial membrane potential and the level of GSH increased by 10% after treatment with Li or Kenpaualone for 24h. Caspases: The basal (low) level of caspase-3 activity was decreased by 20%. No significant effects were found concerning caspase-8 or 9 activities. Calpain and proteasome activity: No effect was observed on the activity of calpain, metalloproteases or the proteasome.

Conclusion Inhibition of GSK-3 may protect against oxidative damage and attenuate apoptosis in HLEC and in the mouse lens in organ culture. No changes of the major proteolytic systems in the cell were detected.

■ 2334

Studies on nitric oxide generation and Na,K-ATPase inhibition in porcine nonpigmented ciliary epithelium

DELAMERE NA (1, 2, 3), SHAHIDULLAH M (1, 3)

(1) Department of Ophthalmology & Visual Science, Univ. Louisville, Louisville, KY

(2) Department of Pharmacology & Toxicology, Univ. Louisville, Louisville, KY

(3) Department of Physiology, Univ. Arizona, Tucson, AZ

Purpose Nitric oxide (NO) donors can reduce the rate of aqueous humor formation (Shahidullah et al., 2005). To examine the mechanism of action, studies were conducted to determine whether the nonpigmented ciliary epithelium (NPE) is able to generate NO and to examine the effects of NO donors on Na,K-ATPase activity in the NPE.

Methods NPE cells were isolated from pig eyes and incubated with test compounds. The appearance of nitrite in the incubation solution, an indicator of NO production, was detected by the Griess assay. After incubation, the NPE was homogenized and Na,K-ATPase activity was measured using a colorimetric method.

Results The presence of NO synthase in the NPE was determined by immunolocalization. Exposure of the NPE to L-arginine, a physiological precursor of NO, or the NO donor SNP resulted in the appearance of nitrite in the incubation medium, confirming the ability of the NPE to generate NO. Inhibition of Na-K-ATPase was observed in cells exposed to L-arginine. The L-arginine response was abolished when NO synthase was inhibited by L-NAME. Exposure of the NPE to the NO donor SNP also caused inhibition of Na,K-ATPase. The inhibitory effect of SNP on Na,K-ATPase activity was abolished by the soluble guanylate cyclase inhibitor, ODQ, suggesting the involvement of cGMP. Na,K-ATPase activity was inhibited in cells treated with 8p-CPT-cGMP, a cell permeable analog of cGMP. The inhibitory effect of 8p-CPT-cGMP on Na,K-ATPase was abolished by selective protein kinase G (PKG) inhibitors, H-8 and H-9.

Conclusion The NPE cell layer is capable of NO generation. NO appears to inhibit Na,K-ATPase activity by a mechanism that involves activation of soluble guanylate cyclase, generation of cGMP and activation of PKG.

■ 2335

Age-related changes in the orbital layer of human extraocular muscles (EOMs)

BRUENECH JR, KJELLEVOLD HAUGEN IB

Biomedical research unit, Dep. of optometry and visual science, Buskerud University College, Kongsberg

Purpose Recent studies have shown that the neuromuscular architecture of EOMs go through several age-related changes. These alterations are believed to have functional implications, yet information regarding the affected fibres and their distribution is rather limited. Many orbital fibres have been found to insert into collagenous structures referred to as muscle sleeves or pulleys. In view of the controversy concerning double insertions of rectus muscles, knowledge regarding aging processes of these fibres is of particular interest. The purpose of this study was hence to analyse the neuromuscular arrangement of EOMs, with emphasis on orbital fibres, and thereby contribute to the understanding of ocular motility.

Methods Medial rectus muscles from 5 humans (3-90 years), with no history of neuromuscular disease or binocular anomaly, were prepared for light and electron microscopy using standard histochemical techniques. Transverse serial sections were obtained from the middle and distal third of the muscles.

Results The muscle fibre types of the orbital layers were consistent with previous reports. A significant number of Fibrillenstruktur fibres from mature subjects were found to be served by more than one motor endplate. Loss of myofilaments, accumulations of lipofuscin and other forms of degenerations were also present in these specimens.

Conclusion Accepting the notion that orbital fibres can influence the insertions of rectus muscles, the current study shows that such an effect must arguably decline with age. The reported alterations may hence be a contributing factor in the reduced oculomotor capacity observed in elderly. The absence of both multiple and polynerval innervation in infants supports this view.

■ 2336 / 201

Ectasia After LASIK; Case Report

ABAHUSSIN M (1), VELARDE J (2), MEEK KM (1), COTERO J (2)

(1) School of Optometry and Vision Sciences, Cardiff University, Cardiff

(2) The Cantabrian Institute of Ophthalmology, Spain

Purpose To study the ultra-structural features of a post-LASIK ectatic cornea from a low myopic patient.

Methods X-ray diffraction (XRD) was used to study collagen fibril orientation within the stroma of a normal cornea and a grafted 7mm post-LASIK ectatic corneal button with a residual stromal bed thickness of about 350 microns. Scanning electron microscopy (SEM) and light microscopy (LM) was used to further characterise the ultra-structural features of the ectatic cornea.

Results The normal preferred orthogonal orientation of collagen fibrils in the superior-inferior and nasal-temporal directions is unaltered in post-LASIK ectatic tissue. SEM revealed a thinning and unknown defect at the inferior part of the cornea. Moreover, during SEM preparation, the LASIK-flap separated from the remaining cornea thus indicating that after four years, the flap still does not contribute to biomechanical integrity of the cornea.

Conclusion Post-LASIK ectasia is not associated with any change in collagen orientation. Tissue thinning in post-LASIK ectasia occurs only within the intact stromal bed; flap thickness remains unaltered. The detachment of the flap from the stromal bed during SEM processing, which utilises chemicals known to cause cellular disruption, suggests that cellular rather than matrix components are involved in flap adhesion.

■ 2337 / 202

Metabolic and Molecular Signalling Pathways of Retinoids on Human Trabecular meshwork

SAPIN V (1, 2), BLANCHON L (1), MARCEAU G (1, 2), NEZZAR H (3, 1), DASTUGUE B (2), RIGAL D (3, 1), YUE B (4), CHIAMARETTA F (3, 1)

(1) ARDEMO, Clermont Ferrand

(2) INSERM U.384, Clermont Ferrand

(3) Department of Ophthalmology, Clermont Ferrand

(4) University of Illinois, Chicago

Purpose The integrity of the human ocular surface has an absolute requirement of vitamin A and its active derivatives, the retinoic acids. These retinoids act through the activation of nuclear receptors: RARs and RXRs. These receptors have been studied in rabbit, mouse and human cornea, but tissue and cellular expression of the 3 RARs and 3 RXRs had to be established at the human trabecular meshwork. The first objective of this work was to define their expression patterns in term of genes and proteins. The second objective was to demonstrate the presence of different enzymes transforming vitamin A to retinoic acid and the activity of this retinoids pathway in the ocular surface.

Methods Total mRNA was extracted from total human trabeculum meshwork, primary cell cultures of human trabecular meshwork established from donor corneas, and submitted to RT-PCR experiments. Immunological staining experiments were performed to better define the RARs and RXRs protein localization. The expression patterns of retinal dehydrogenase (RALDHs) and alcohol dehydrogenase (ALDHs) was also determined in human trabecular meshwork cells. The metabolic pathway is tested by targeted disruption of different enzyme steps transforming retinol to retinoids.

Results RAR alpha, beta, gamma, RXR alpha, ALDH4 and RALDH2 are expressed in trabecular meshwork. Trabecular cell meshworks are able to convert retinol into retinoic acid.

Conclusion For the first time, we established an exhaustive description of RARs and RXRs, ALDHs and RALDHs expression patterns, in the human trabecular meshwork. Our results demonstrated in the human trabecular meshwork, the presence of all the actors of retinoic acid signalling pathway and their activation.

■ 2341

Mapping of the age-related EMORY cataract: indication for a complex trait

GRAW J, WEBER A, LÖSTER J

GSF-National Research Center for Environment and Health, Institute of Developmental Genetics, Neuherberg

Purpose The EMORY cataract is one of the very few mouse models for hereditary senile cataracts. Aim of the actual study is the mapping of the mutation to further identify candidate genes within the critical linkage intervals.

Methods EMORY mice were imported from the Jackson Laboratory into the GSF animal facilities in 2003. Homozygous EMORY mice were outcrossed to wild-type Japanese fancy mice (JF1). Mice were analyzed for cataracts using slit lamp at the age of 9 months; since no carriers were observed among the F1 generation, only the backcross of these heterozygotes to homozygous EMORY mice was further considered for the genome-wide linkage analysis using a panel of microsatellite markers.

Results 482 F2 animals have been collected; surprisingly, 110 of them died within the first few months (71% males, 29% females). The remaining 386 F2 offspring (between 4 and 9 months old) have been investigated for lens opacities, and finally 68 carriers have been confirmed (67% albino, 37% agouti). The age of onset was shifted to younger animals, mainly 4–5 months of age (~1/3 of the carriers). Significant linkage was found to chromosomes 5, 7, 12 and 19. Therefore, it is obvious that the EMORY cataract does not fulfill the classical criteria for a Mendelian disorder; moreover, it has to be considered as a complex trait. On chr. 5, the Cryb-gene cluster has been excluded because of its position; the same is true on chr. 7 for Lim2 and Six5. Some other candidate genes will be discussed (e.g. Fgfbp on chr. 5; FoxG1 on chr. 12 and Pitx3 on chr. 19).

Conclusion The EMORY cataract in the mouse is a complex disorder with several genes involved.

■ 2342

Removal of retrobulbar cysticercus cellulosae

BARRAQUER J

Centro de Oftalmología Barraquer, Barcelona

Purpose Ocular implantation of "Cysticercus cellulosae," the larval stage of tenia solium is rare in Europe. It may be localized subretinally or in the vitreous, occasionally in the anterior chamber, the choroid or the iris. Ophthalmoscopically the parasite is identified as a cystic formation, maximum diameter of 15 mm., with characteristic brilliant coloured walls. Occasionally the peristaltic movements and the body of the cysticercus can be seen. Removal "in toto" is imperative as spontaneous or traumatic rupture of the "cyst" would lead to severe inflammatory reaction and irreparable definite loss of the eye.

Methods To illustrate these concepts the removal of a retrobulbar "Cysticercus cellulosae" in a 35-year-old patient, seen in our Clinic in 1962, is illustrated. The parasite is clearly identified behind the transparent crystalline lens. A large, 180° incision is performed and the lens removed intracapsularly, after application of alphachymotrypsin solution (1:5000) (enzymatic zonulolysis, Barraquer 1958). A limited hyaloidotomy is performed and the parasite with the surrounding cyst is displaced forward, "in toto," using gentle expression maneuvers and then removed with a spoon and marten hair brush without any vitreous loss.

Results After the removal of the cyst, the perfectly visible movements of the encapsulated parasite clearly demonstrate that this was a live cysticercus cellulosae and the eye had been saved by the surgical intervention performed without delay.

Conclusion In spite of the high surgical risk removal "in toto" of the parasite and surrounding cyst and previous extraction of the crystalline lens is justified as the only option to save the eye in these cases.

■ 2343

Binocular vision and refractive results of the secondary IOL implantation in children with long-term follow-up

AUTRATA R, PYROCHTOVA S, REHLÍREK J

Department of Pediatric Ophthalmology, Masaryk University Hospital, Brno

Purpose To evaluate functional outcomes of techniques for implanting intraocular lenses (IOL) in aphakic children in the absence of adequate capsular support and for placing a secondary IOL within the capsular bag.

Methods 68 children, aged from 36 months to 16 years (mean 7.95 years), who originally had aphakia after cataract extraction were operated from 1995 to 2003. The mean follow-up time was 6.9 years (range: 3 to 10 years). Visual outcomes, refraction, and complications of 93 eyes were reviewed. Secondary PC IOL implantation with transscleral fixation was performed in 25 eyes, anterior chamber (AC) IOLs in 17 eyes in the absence of capsular support. 19 eyes had PCIOL implantation within the reopened capsular bag. In 32 eyes PC IOLs were implanted into the ciliary sulcus without suture fixation.

Results The best spectacle corrected visual acuity (BSCVA) postoperatively was preserved at 55 eyes, improved at 38 eyes in comparison to their preoperative value. Uncorrected visual acuity (UCVA) was improved at all eyes. The mean SE refraction preop. was +13.28 D and improved to +1.14 D postop. Binocular vision status was maintained at 57 children and improved at 11 children. High grade stereopsis was present at 29% of children with bilateral pseudophakia and at 10.4% of children with unilateral pseudophakia. The highest complication rate was observed after AC IOL implantation cases. Posterior chamber IOLs implanted into the reopened capsular bag as well as transscleral suture fixated PC IOLs induced significantly less complications.

Conclusion Secondary PC IOLs implantation is a safe and effective method for correction of pediatric aphakia, especially in the bag-IOL implants.

■ 2344 / 227

Evolution of Ultraviolet Radiation-B (UVR-B)-Induced Cataract in the Pigmented Guinea Pig

MODY VC, KAKAR M, LÖFGREN S, AYALA M, MEYER L, DONG X, WANG J,

BUCHT C, SÖDERBERG PG

Karolinska Institute/St. Erik's Eye Hospital, Stockholm

Purpose To investigate the short-term development of cataracts after acute exposure to ultraviolet radiation-B (UVR-B) in the pigmented guinea pig.

Methods Twenty-four female pigmented guinea pigs, five to eight weeks of age, were exposed unilaterally to 80 kJ/m² UVR-B under anesthesia *in vivo* for sixty minutes. The guinea pigs were euthanized after post-exposure intervals of 1, 2, 4, or 8 days. Each lens was extracted and photographed. The development of forward light scattering for the lens was used to quantify the amount of cataract as expressed in transformed equivalent diazepam concentration units (tEDC).

Results All non-exposed lenses were devoid of cataract. All exposed lenses developed anterior subcapsular opacities at 1 day after UVR-B exposure. The surface area of the opacities decreased from 2 days to 8 days after exposure. Exposed lenses scattered more light than the corresponding contralateral lenses for the animals of the four groups. The mean differences ± 95% confidence intervals in forward light scattering between the exposed and non-exposed lenses for each group in tEDC were: 0.052 ± 0.036 [1 day]; 0.034 ± 0.018 [2 days]; 0.041 ± 0.024 [4 days]; and 0.044 ± 0.018 [8 days]. A maximum amount of forward light scattering in the exposed lens developed at one day post-UVR-B-exposure and was still present up to eight days after exposure.

Conclusion Anterior subcapsular cataract develops one day after exposure to UVR-B in the pigmented guinea pig. Maximum intensity of forward light scattering develops one day after exposure to UVR-B and remains constant up to eight days after exposure. One day is an appropriate time interval for study of UVR-B safety limit in the pigmented guinea pig.

■ 2345 / 228

**A Phacoemulsification Anticipation of Difficulty (PAD):
Anterior chamber depth predicts difficulty of surgery**

SIDDIQUI S (1), SOLEBO L (1), HAYWARD JM (1), KERR-MUIR M (2), GALE RP (1)

(1) Department of Ophthalmology, York Hospital, York

(2) Department of Ophthalmology, Addenbrookes Hospital, Cambridge

Purpose The training of ophthalmologists to perform cataract surgery is becoming an increasingly contentious issue. Studies have shown that 37% of cataract operations are performed by those at specialist registrar level or below. It is imperative that cases of appropriate difficulty are selected throughout the training of cataract surgeons. We designed a 10-point user-friendly scale to anticipate difficulty of phacoemulsification cataract surgery at pre-operative assessment for use as a tool for the trainee ophthalmologist.

Methods 84 patients were studied over a 4-month period following COREC and research and development approval. Data was collected using a standardised preoperative scale. Perceived post-operative difficulty was scored on a visual analogue scale by 3 experienced consultant surgeons, blinded to the pre-operative score.

Results There was no difference in inter-observer variability for pre-operative scoring ($p<0.05$). A strong correlation between the pre-operative grading of the anterior chamber depth (either deep or shallow) and perceived difficulty of surgery is demonstrated ($p<0.05$). This indicated that AC depth was the most powerful predictor intraoperative difficulty.

Conclusion The PAD scale ensures thought is given to the anticipated difficulty of cataract surgery prior to case selection for ophthalmologists in training.

■ 2346 / 229

Cataract and inflammation: a new drug delivery system

EPERON S (1), BOSSY-NOBS L (2), GUEX-CROISIER Y (1), PETROPOULOS IK (1),

GURNY R (2)

(1) Jules Gonin Eye Hospital, University, Lausanne

(2) Department of Pharmaceutics and Biopharmaceutics, University, Geneva

Purpose Cataract surgery is often performed in patients suffering from other pathologies, which imply high doses of steroids via oral or injection route. Moderate efficacy, risks and secondary effects are often observed. Thus it is of great importance to develop a biodegradable drug delivery system (DDS) associated to the artificial intraocular lens (IOL).

Methods DDS were manufactured using poly(D,L-lactide-co-glycolide) as matrix. The amount of triamcinolone acetonide (TA) loaded in DDS was evaluated as well as the release profile of TA. Cataract surgery was performed on the right eye of pigmented rabbits followed by DDS insertion. Inflammation parameters were: i) the clinical score (sum of hyperemia, chemosis, edema and secretion, each scored from 0 to 3), ii) the number of inflammatory cells and iii) the protein concentration in aqueous humor (AH).

Results The DDS weighed 2.94 ± 0.08 mg and measured 2.1 ± 0.2 mm. The loading capacity was approximately of 338 ± 7 microg of TA per mg of polymer. Comparisons were made between rabbits wearing i) no DDS, ii) unloaded DDS, iii) loaded DDS, iv) two loaded DDS. The in vivo investigations showed a good ocular biocompatibility. Loaded DDS could significantly reduce ocular inflammation induced by surgery, especially concentration of inflammatory proteins within AH.

Conclusion The results suggest that our type of DDS loaded with TA would be a promising system for the delivery of steroids to reduce ocular inflammation.

■ 2347 / 230

Ocular Anaesthetic Scoring System: Does the use of hyaluronidase in ocular anaesthesia improve patient satisfaction?

CEHAJIC J (1, 2), WEARNE IM (2)

(1) West Kent Eye Centre, Princess Royal University Hospital, Bromley Hospitals NHS Trust, London

(2) Eastbourne District General Hospital, Eastbourne

Purpose To use Ocular Anaesthetic Scoring System (OASS) to compare effectiveness of anaesthetic techniques and to determine whether this correlates with patient satisfaction of non-topical anaesthesia during cataract surgery.

Methods We developed OASS as a quick means of assessing non-topical anaesthesia. A score between 0 (poor) and 14 (excellent) is obtained using objective (degree of ptosis, akinesia and orbicularis function) and subjective measures (digital spear pressure at limbus and topical anaesthetic sting). 75 patients were randomised to receive either sub-Tenon's (ST) or peribulbar (PB) block. The ST group was further divided into a low (150 units) or high (300 units) level of hyaluronidase group. Anaesthesia was assessed immediately preoperatively using OASS. Postoperative patient satisfaction was determined by Visual Analogue Pain Scale (VAPS) and Iowa Satisfaction with Anaesthesia Scale (ISAS). Statistical analysis was performed using non-parametric tests.

Results Using OASS the ST approach achieved significantly better scores than the PB ($P<0.001$). Higher levels of hyaluronidase (300 units) were associated with significantly higher OASS scores ($P<0.001$). Spearman rank correlation showed that OASS correlates significantly with well-established subjective measures, VAPS (-0.60, $p<0.0001$) and ISAS (0.52, $p<0.0001$). The median satisfaction score was significantly higher in the ST than in the PB group and in the high level compared to the low level hyaluronidase group.

Conclusion The OASS has been shown to be an effective tool in assessing and comparing non-topical anaesthesia and techniques. Furthermore, a patient who has had a good block, as defined by OASS, reports significantly higher satisfaction scores with anaesthesia during cataract surgery.

■ 2351

Dynamic Contour Tonometry – the new „gold standard“ for intraocular pressure measurement?

PACHE M

University Eye Hospital, Freiburg

Purpose Dynamic contour tonometry (DCT) with the PASCAL device offers the possibility to measure intraocular pressure (IOP) largely independent of corneal properties. We a) compared DCT with Goldmann applanation tonometry (GAT) in eyes with normal corneas. In addition, we b) compared the PASCAL handheld with Perkins and Tonopen in the supine position.

Methods We prospectively measured IOP a) using DCT and GAT in random order in 100 right eyes of 100 subjects in the upright position, and b) using DCT, Perkins and Tonopen in 76 right eyes of 76 subjects in the supine position. The agreement between the methods was tested by Bland-Altman-Analysis (BAA).

Results a) BAA demonstrated a mean difference GAT-DCT of -1 mmHg, b) Mean IOP values was 20.1 mmHg for Tonopen (19.7 mmHg after correction for CCT), 19.0 mmHg for DCT and 16.1 mmHg for Perkins. The difference between Perkins and both Tonopen and DCT was statistically significant ($p<0.0001$). Mean CCT value was 557 (range: 420 – 652) μ m. BAA showed a mean deviation of 1.05 mmHg between Tonopen and DCT, of 3.95 mmHg between Tonopen and Perkins and 2.89 mmHg between DCT and Perkins. None of the tonometers showed a significant CCT-dependency of IOP values.

Conclusion We found a) a good agreement between DCT and GAT, and b) between DCT and Tonopen values, but not between Perkins and DCT and Tonopen, respectively. In none of the 3 devices, a dependency of IOP-values on CCT was observed. Our study is limited by the fact that no comparison with intracameral IOP readings was performed. Until data from ongoing studies comparing DCT with intracameral IOP values are available, none of the three devices can be considered superior to the others.

■ 2352

Technical Principle of Dynamic Contour Tonometry compared with Goldmann Applanation Tonometry

LANGENBLICHER A

Institute of Medical Physics, University Erlangen-Nuremberg, Erlangen

ABSTRACT NOT PROVIDED

■ 2353

Clinical Impact of Dynamic Contour Tonometry - a new state of the art?

VIESTENZ A

Dept of Ophthalmology, University Magdeburg, Magdeburg

ABSTRACT NOT PROVIDED

■ 2354

Dynam-Ico - DCT versus intracameral manometry

LAUTEBACH S, NEUBLIGER M, FUNK J

University Eye Hospital Freiburg, Freiburg

Purpose The Dynamic Contour Tonometry (DCT) is a new method to determine IOP. Several studies have shown a good reliability and validity of DCT in comparison to Goldmann applanation tonometry (GAT). Different from GAT, it is supposed to be largely independent from central corneal thickness (CCT). To confirm this, we compared DCT measurements with direct intracameral IOP readings. The intracameral IOP readings were taken with a DCT tip.

Methods Thus far, 10 patients scheduled for cataract surgery were included in the study. We simultaneously recorded 1) manometric IOP as determined by an infusion bottle connected to the anterior chamber, 2) intracameral IOP recorded by a pressure sensor, 3) DCT with the handheld version of the device. IOP was adjusted to 10, 20 and 30 mmHg by varying the height of the infusion bottle. CCT was measured by Ultrasound.

Results We found a good correlation between intracameral pressure and the results of DCT (correlation coefficient of 0.97). Bland Altman analysis revealed a mean difference of $1.03 \text{ mmHg} \pm 2.4 \text{ mmHg SD}$. The difference showed a non significant tendency to increase with higher IOP values. No correlation between IOP values and CCT was observed.

Conclusion Our preliminary results suggest that DCT shows a good agreement with direct intracameral IOP values.

■ 2355

Correlation between OPA and CDI in glaucoma patients versus normal subjects

STALMANS I (1), ZEYEN T (1), HARRIS A (2)

(1) Dept of Ophthalmology, University Hospital Leuven, Leuven

(2) Dept of Ophthalmology, Indiana University School of Medicine, Indianapolis

Purpose 1) To compare Ocular Pulse Amplitude (OPA) between healthy subjects and patients with normal tension or high tension glaucoma. 2) To study a putative correlation between OPA and flow velocity parameters as measured by Color Doppler Imaging (CDI).

Methods Three groups of individuals were defined: patients with normal tension glaucoma or primary open angle glaucoma, and non-glaucomatous individuals. Color Doppler Imaging (CDI) of central retinal artery, short posterior ciliary arteries and ophthalmic artery, as well as OPA measurement, were performed twice with one month interval. The mean values between the two visits were calculated. These average values were used to calculate any correlations between OPA and retrobulbar blood velocities.

Results 1) OPA was significantly reduced in patients with primary open angle glaucoma ($p=0.007$) as well as normal tension glaucoma patients ($p=0.000003$), as compared to non-glaucomatous individuals. There was no significant difference in OPA between the two patients with low tension or high tension glaucoma ($p=0.2$). 2) A systematic correlation was observed between the OPA on the one hand and the systole/diastole ratio as well as the resistive index on the other hand in all measured vessels in the healthy individuals (p -values varied between 0.03 and 0.003). This correlation was much less evident in the glaucoma patients.

Conclusion OPA was significantly reduced in normal tension as well as high tension glaucoma patients. Although OPA and CDI parameters strongly correlated in the healthy individuals, this was not obvious in the glaucoma patients. Possible pathogenetic mechanisms and clinical implications will be discussed.

■ 2361**Gene Therapeutic Prospects in EOSRD***ROLLING F**INSERM U649, Nantes*

Purpose The purpose of our study was to evaluate rAAV2 pseudotyped with serotype 4, a serotype which specifically targets the retinal pigmented epithelium, for ability to restore vision in RPE65^{-/-} purebred Briard dogs and to assess the safety of gene transfer with respect to retinal morphology and function.

Methods Recombinant rAAV2/4 and rAAV2/2 vectors containing the human specific RPE65 promoter and cDNA were generated and administered subretinally in 8 affected dogs, ages 8 to 30 months (n=6 with rAAV2/4, n=2 with rAAV2/2).

Results Electrophysiological results demonstrated that restoration of rod and cone photoreceptor function started as soon as 15 days post-injection, reaching maximal function at 3 months post-injection, and remaining stable thereafter in all animals treated at 8 to 11 months of age. As assessed by the ability of these animals to avoid obstacles in dim light, functional vision was restored in the treated eye, while the untreated contralateral eye served as an internal control. The dog treated at a later age (30 months) did not recover retinal function or vision, suggesting the existence of a therapeutic window for the successful treatment of RPE65^{-/-} dogs by gene replacement.

Conclusion We have demonstrated herein that specific gene transfer to the RPE using a rAAV2/4 vector can be used to correct the RPE65 defect in RPE65^{-/-} purebreed Briard dogs, leading to the stable restoration of normal retinal function and vision dependent behavior. The relative contributions of dysfunction or cell death to the extreme visual loss in human RPE65 deficiency are unknown. However analysis of our results in dogs suggests that for successful gene therapy to occur in humans, patients should be treated at a young age.

■ 2362**Retinoblastoma Update**

DESJARDINS L (1), LUMBROSO-LEROUIC L (1), LEVY-GABRIEL C (1), DOZ F (2), DENDALE R (3), SASTRE X (4), BOURS S (5), ASSELAIN B (5)

(1) *Ophthalmology, Paris*

(2) *Pediatric, Paris*

(3) *Radiotherapy, Paris*

(4) *Pathology, Paris*

(5) *Biostatistics, Paris*

Purpose To describe the efficacy of conservative management of retinoblastoma (tumor control, decreased use of external beam radiotherapy) by the combination of two cycles of carboplatin and etoposide chemotherapy followed by local therapy, including chemothermotherapy (intravenous carboplatin followed by thermotherapy to the tumor). Progresses in orbital radiotherapy using iodine lines will also be described. Future directions for research will be reviewed.

Methods 83 children were included in a prospective study ; 19 had unilateral lesions (22.8%) and 64 (77.1%) had bilateral lesions. 115 of the 147 affected eyes were eligible for conservative management. 66 children received neoadjuvant chemotherapy before local therapy, which consisted of one or a combination of several techniques: chemothermotherapy (65 children; 86 eyes) with a mean of 3 cycles per child, thermotherapy alone (22 children; 24 eyes), cryoapplication (49 children; 58 eyes), and 125 plaque brachytherapy (26 children; 29 eyes).

Results These various combinations achieved tumor control for 97 eyes (84%). External beam radiotherapy was necessary for 9 children (11%) 13 eyes (12%) and constituted initial treatment for 7 children (10 eyes). Enucleation was necessary for 23 eyes (20%), because of complications in 5 cases. In children treated by primary enucleation who present histological risk factors with the need for orbital radiation, satisfactory cosmetic result could be achieved by iodine 125 brachytherapy.

Conclusion neoadjuvant chemotherapy with two cycles of carboplatin and etoposide followed by local therapy, including chemothermotherapy, ensures satisfactory tumor control and decreases the use of external beam radiotherapy.

■ 2363**Retinopathy of Prematurity Update***LORENZ B*

Dept. of Paediatric Ophthalmology and Ophthalmogenetics, Klinikum, University of Regensburg, Regensburg

Purpose News from the World ROP Meeting in Vilnius, 14-16 september 2006.

■ 2411

Ophthalmological data of 837 patients with FABRY disease: latest analysis from FOS – the Fabry Outcome Survey

PITZ S (1), IOANNIDES A (2), SODI A (3), SLUNDER-PLASSMANN G (4)

(1) Ophthalmology, Mainz

(2) Ophthalmology, London

(3) Ophthalmology, Florence

(4) Nephrology, Vienna

Purpose Fabry disease is an X-linked lysosomal storage disorder caused by deficient activity of the lysosomal enzyme alpha-galactosidase A. Progressive accumulation of the enzyme substrate in cells throughout the body leads to organ failure and premature death. Treatment has recently become available in the form of enzyme replacement therapy. To determine the efficacy and safety of ERT with agalsidase alpha, FOS – the Fabry outcome survey – has been established. The present analysis of the FOS database aims to provide ophthalmological data on the natural history of Fabry disease.

Methods The FOS database was analysed in terms of ophthalmological findings in Fabry disease.

Results As of March 2006, 837 patients were enrolled. For 246 of these (126 female, 120 male), detailed ophthalmological data were available. While the sex distribution of the ophthalmological hallmark of the disease, the cornea verticillata, was equal (69% of the males and 73% of the females affected), this did not hold true for the other ophthalmological features. 41% of the males show either conjunctival or retinal vessels tortuosity, while this sign was found in 25% of the affected female patients. Likewise, only 20% of the male Fabry patients suffered from the typical Fabry cataract, while this peculiar form of posterior lens opacification was noted in 7% of female patients.

Conclusion FOS is providing valuable information on the prevalence of ophthalmological signs in the to date largest data base on this orphan disease. Since the advent of enzyme replacement therapy, early diagnosis is of utmost importance. The high prevalence of cornea verticillata in FOS emphasises the value of ophthalmological screening for this disease.

■ 2412

Novel mutations in ABCR gene associated with Stargardt Disease in Italian patients

SODI A (1), PASSERINI I (2), GIAMBENE B (1), MENCHINI U (1), TORRICELLI F (2)

(1) Department of Ophthalmology, Careggi University Hospital, Florence

(2) Department of Genetic diagnosis, Careggi University Hospital, Florence

Purpose Stargardt disease (STGD) is a juvenile-to-young adult onset progressive macular degeneration, which is predominantly inherited as an autosomal recessive trait. Mutations in the ABCR gene, encoding a photoreceptor-specific transporter, have been associated with the disease. In this study we determined the mutation spectrum in the ABCR gene in a group of Italian patients with autosomal recessive Stargardt disease.

Methods Fifty families from central Italy, some members of which were affected by autosomal recessive Stargardt disease, were clinically examined. DNA samples were analyzed for mutations in all 50 exons of the ABCR gene by DHPLC approach (with optimization of DHPLC conditions for mutation analysis) and direct sequencing techniques.

Results In all the affected patients we reported some mutations of ABCR gene. Some of these mutations have been already described and among them G1961E was the most frequent in our series. Twenty-two mutations had not been previously described, and were not detected in 150 unaffected control individuals (300 chromosomes) of Italian origin.

Conclusion Some novel mutations in ABCR gene in STGD patients were reported. These data confirm the extensive allelic heterogeneity of the ABCR gene. The frequent report of novel mutations is probably related to the great number of exons in the ABCR gene, which favours rearrangements in DNA sequence.

■ 2413

A novel homozygous NR2E3 mutation in a patient with enhanced S-cone syndrome

ROCHA DE SOUSA A (1, 2), HAYASHI T (3), BRANDAO E (1), GOMES N (1), PENAS S (1), MARQUES CJ (4), TAKEUCHI T (3), FALCAO-REIS F (1, 5)

(1) Dept Ophthalmology S João Hospital, Porto

(2) Dept Physiology Faculty of Medicine, University of Porto, Porto

(3) Department of Ophthalmology, Jikei University School of Medicine, Tokio

(4) Department of Genetics, Faculty Of Medicine University of Porto, Porto

(5) Dept Ophthalmology Faculty of Medicine, University of Porto, Porto

Purpose To investigate the ophthalmic features of a severe form of enhanced S-cone syndrome in a 25 years old Portuguese patient. A genetic analysis had been performed.

Methods Fundus examination and angiography, Goldman visual fields perimetry (VF), color vision tests, Vistech contrast sensitivity, full field and s cone electroretinography (ERG), optical coherence tomography (OCT), were performed. Mutation screening for NR2E3 gene was performed with polymerase chain reaction and direct sequencing.

Results The patient had visual acuity of 5/10 in OD and 3/10 in OS, normal color vision, normal visual fields and normal contrast sensitivity. Fundoscopy and angiography showed mild degenerative lesions in the midperipheral retina without macular leakage. Patient had an absence of scotopic response with a reduced and profound delayed photopic and combined response, and a residual flicker response in the ERG. In the s cone ERG the L-M b wave was absent. A cystoid-like degeneration of the macula was observed in both eyes on the OCT. Mutation analysis identified a homozygous missense mutation (Cys83Tyr) in the DNA binding domain.

Conclusion Our study identifies a new homozygous NR2E3 mutation in a patient with a severe form of enhanced S cone syndrome.

■ 2414

Screening for Sequence Changes in the OPA1 gene in 21 patients with Optic Atrophy

CARTER JG, CHURCHILL AJ

Ophthalmology, University of Bristol, Bristol

Purpose We aimed to assess the potential cause of Optic Atrophy in 9 families and 11 simplex cases by screening for mutations in the OPA1 gene, the most common loci for pathogenic genetic changes

Methods Clinical examinations supported diagnosis of optic atrophy in all cases, although considerable variability in phenotype was seen. DNA was extracted and exons 1-28 of OPA1 were amplified via PCR. These amplicons were assessed for variation by heteroduplex analysis using the Transgenomic WAVE system, in comparison with a wildtype sequence. Any variations from wildtype in resultant chromatograms were defined by direct sequencing

Results 8 different mutations were discovered, 2 of which were novel. Both novel mutations cause truncation of the OPA1 protein; exon 19 - 1828C>T (Gln610Stop), and exon 26 - 2703delT (Glu902fsStop906). We also uncovered 13 single nucleotide polymorphisms (SNPs), of which 3 were novel (Intron 1, 33-8>c; Intron 9, 984+35g>t; Intron 11, 1141-20g>t)

Conclusion We detected OPA1 mutations in 2/12 simplex cases (17%) and 6/9 families (67%). A further 1/9 familial and 5/12 simplex cases exhibited polymorphisms. One familial case showed incomplete penetrance, highlighting the difficulty in clinical diagnosis. Our relatively low simplex pick-up rate may be attributed to the wide range of optic atrophy phenotypes, some of which may be non-genetic. Our screen has allowed us to successfully counsel 8 families/individuals about the cause of their optic atrophy and discuss implications for other family members

■ 2415

Is the clinical picture of Leber hereditary optic neuropathy changing?

WEFERS BETTINK - REMEIJER M (1), SPRUIJL T L (2)

(1) Neuro-ophthalmology Department Het Oogziekenhuis Rotterdam, Rotterdam
(2) Department of Genetics and Cell Biology University of Maastricht, Maastricht**Purpose** To report a series of patients with a late onset of Leber Hereditary Optic Neuropathy**Methods** A series of nine patients with a (sub) acute bilateral loss of vision, caused by late onset of Leber Hereditary Optic Neuropathy (LHON)**Results** Age of onset in these patients varied from 48-73. (mean 61.4) 7 male and 2 female patients. Visual Acuity varied from HM to 0.1. Four had a positive family history for LHON. None had a history of alcohol abuse, but most of them were "social" drinkers. Four of the patients were heavy smokers (1 female). None of the patients had a Vitamin B 12 deficiency. There was no clue for toxic optic neuropathy or other deficiencies. Mutations found were mainly two of the three primary mitochondrial DNA mutations: 11778G>A, 3460G>A**Conclusion** Nine patients with a (sub) acute bilateral loss of vision had a late onset Leber Hereditary Optic Neuropathy. Mean age 61.4. Usually LHON develops earlier in life with a peak in the age of 15-35.

■ 2416 / 374

Mutation analysis of RHO gene and novel mutation Arg252Pro (755G→C) in patients with nonsyndromic retinitis pigmentosa from BashkortostanGRINBERG E, DZHEMILEVA L, KHUSNUTDINOVA E
Institute of biochemistry and genetics, Ufa**Purpose** To identify mutations in the RHO gene in patients with nonsyndromic retinitis pigmentosa (RP) from Bashkortostan. RP is a clinically and genetically heterogeneous group of retinal degenerative diseases. The prevalence of RP is 19 to 27 per 100000 in general population. In the adRP approximately 30% of families have mutations in the RHO gene.**Methods** We examined all exons of RHO gene in 120 unrelated patients with RP and 120 unaffected individuals by single-strand conformation polymorphism analysis (SSCP) and direct sequencing. Patients were examined clinically and with visual function tests.**Results** When the entire RHO gene was examined, we detected sequence change IVS3+4c→t, and its frequency more in patients (0.36) than in controls (0.1). Also we analyzed polymorphism Mfd2CA of RHO gene and revealed 14 allelic variants. Also we report the identification of a novel mutation Arg252Pro (755G→C) of the 4 exon RHO gene.**Conclusion** There were statistically significant differences in allele and genotype frequencies of polymorphism IVS3+4c→t of RHO gene in affected patients with RP and in controls. So, according to our data, this polymorphism is likely to be pathogenic. Alleles 116 and 132 of polymorphism Mfd2CA of RHO gene are significantly more frequent in patients than in controls. Novel mutation Arg252Pro (755G→C) of the 4 exon RHO gene was detected in 1 bashkirian family with RP. All family members carried mutation Arg252Pro in heterozygous form and have similar fundus appearances. Identification of the molecular defects underlying retinal degeneration will allow clinicians to establish more accurate diagnoses and prospects for prenatal diagnostic.

■ 2417 / 375

Familial high myopia in Polish population

RYDZANICZ MA (1), PODFIGURNA-MUSIALAK M (2), FRAJDENBERG A (3), BEJJANI BA (4), LEAL SM (5), GAJECKA M (1, 4)

(1) Institute of Human Genetics Polish Academy of Sciences, Poznan
(2) Department of Ophthalmology, Hospital, Leszno
(3) Department of Ophthalmology, Marcinkowski University of Medical Sciences, Poznan
(4) Washington State University, Spokane
(5) Baylor College of Medicine, Houston**Purpose** Myopia is the most common of all ocular conditions. Although high myopia (myopia in excess of - 6.0 diopters [D]) is far rarer than mild/moderate myopia, the importance of high myopia is significant because the development of high myopia involves anterior-posterior enlargement of the eye, abnormal changes in the eye and frequent detachment of the retina. The etiology of myopia is not known. Both genetic and environmental factors seem to play a role. The aim of our study is mapping and cloning gene(s) responsible for familial high myopia in Polish population.**Methods** We have examined collected blood and purified DNA from 199 individuals from 32 unrelated high myopia families in Poland. As a first step before embarking on a genome-wide screen the preliminary genotyping was conducted in 23 families. Prior to preceding with the targeting genotyping, linkage to markers for Stickler syndrome types I, II and III; Marfan syndrome and juvenile glaucoma were tested. Next, we have examined previously proposed familial high myopia loci [18p11.31 (MYP2), 12q21-23 (MYP3), 7q36 (MYP4), 17q21-23 (MYP5), 2q37, 4q22-4q27 (MYP11), and 10q21.1], and additionally to test the linkage for mild/moderate myopia established in Ashkenazi Jewish families and dizygotic twins, 22q12 (MYP6) and 11p13 (MYP7) were genotyping as well.**Results** Linkage to Stickler syndrome, Marfan syndrome and juvenile glaucoma loci were excluded. Genotyping with well-spaced polymorphic markers of high myopia associated loci revealed no evidence of linkage to any of the candidate genes.**Conclusion** There was no evidence of linkage to any of candidate genes and high myopia loci. Further genetic studies are required, genome-wide screen is in progress.

■ 2421**Opportunities for Research Collaboration and Training at The Wilmer Eye Institute***ZACK DJ**Johns Hopkins University School of Medicine, Baltimore*

Purpose One of Wilmer's major strengths is the depth and diversity of its research programs, and its commitment to research training. Basic science studies utilize the latest technologies related to gene therapy, RNA interference, and microarrays; patient-oriented work explores ways of improving the design of clinical trials. Some recent highlights: The Retina Division has a number of active basic science and clinical studies, focusing on mechanisms of angiogenesis as it relates to AMD, proliferative diabetic retinopathy, vein occlusions, and retinopathy of prematurity. The Neuro-Ophthalmology Division is working on a primate model of ischemic optic neuropathy that will be used to test drugs that would either prevent or treat human NAION. The Cornea Division is interested in the genetics of corneal disease and the changing incidence of enophthalmitis following cataract surgery. The Division of Ocular Immunology is playing a leading role in the Studies of the Ocular Complications of AIDS (SOCA) and Systemic Immunosuppressive Therapy for Eye disease (SITE) trials, and is also studying a murine model of Sjogren's syndrome. The Glaucoma Division is studying the molecular basis of RGC death and assessing various diagnostic laser-based imaging methods. The Dana Center has a wide range of projects, from trachoma in Africa to the effect of visual disability on driving. The Pediatrics Division has designed exciting technology that will aid in the diagnosis of visual disease in children. The Retinal Degeneration and Molecular Biology Units are studying the mechanism of action of neurotrophin-mediated neuroprotection, synapse formation in the retina, regulation of retinal gene regulation, and the biology of ocular stem cells. The Lion's Eye Center is studying quantitative methods of measuring vision disability in patients with low vision, how visual impairments interfere with patients' perception of their environment, and potential visual capabilities of patients with prosthetic vision devices.

■ 2422**Research at Rochester***MERIGAN W**-, Rochester*

Purpose Vision researchers at the University of Rochester pioneered the automated measurement and correction of the eye's wave aberration, which has found exciting applications in vision correction as well as high-resolution retinal imaging. In the domain of vision correction, investigators have worked closely with Bausch and Lomb to produce a wavefront-guided refractive surgery system that can reduce higher order aberrations in the eye, as well as customized contact lenses that can correct severe aberrations such as those of keratoconic eyes. Rochester has also used wavefront technology to pioneer the development of high-resolution retinal imaging devices equipped with adaptive optics. These devices allow the imaging of single cells in the living human eye so that the normal and diseased retina, as well as the efficacy of therapy, can be examined at a microscopic spatial scale noninvasively in human and animal eyes. Examples of the use of adaptive optics imaging include the characterization of the cone mosaics of eyes with normal and abnormal color vision, and eyes with cone-rod dystrophy. More recently, adaptive optics, in combination with other technologies such as fluorescence imaging is proving useful for resolving ganglion cells, RPE cells, and blood flow in the smallest retinal capillaries.

■ 2423**Vision research at the Doheny Eye Institute – University of Southern California***RAO NA**Doheny Eye Institute, Los Angeles*

Purpose To review vision research programs at Doheny, including opportunities for research fellowships and collaboration.

Results Vision research at Doheny is focused in four major areas: retina, cornea, optic nerve, and ophthalmic epidemiology. Retina research includes laboratory studies of the pigment epithelium and animal models of subretinal neo-vascularization. A bio-engineering program includes development of electronic retinal implants ("chip.") The Doheny/USC Ocular Surface Center studies dry eye, auto-immune dacryoadenitis, limbal stem cell identification, and corneal wound healing. Doheny Ophthalmic Epidemiology Center studies include the Los Angeles Latino Eye Study and the Multi-Ethnic Pediatric Eye Disease Study. The Doheny Center for Optic Nerve Studies targets an experimental optic nerve crush injury model and a large scale epidemiologic study of Leber's hereditary optic neuropathy. Inflammation/immunology is increasingly important in macular degeneration, limbal stem cell transplantation and dry eye disease. The Doheny Ophthalmic Pathology Laboratory emphasizes immuno-pathology studies of these disorders. Research fellowships, including the opportunity to observe in the Doheny Clinics and Operating Rooms, are available on an individual basis. Limited funds are available to support international fellows. In general, funding is provided by the "home" country of the fellow.

Conclusion A vigorous vision research program at the Doheny Eye Institute in Los Angeles provides opportunities for international collaboration as well as fellows and scholars.

■ 2431

Leukocoria: What is hidden behind a white pupil

HADJISTILIANOU T, DE FRANCESCO S
Ophthalmology, Ocular Oncology Unit, Siena

Purpose The term "Leukocoria" was introduced in 1950 and literally means "white pupil". By definition, cataract is not included.

Methods Review of personal series of cases - 25 years of experience.

Results Leukocoria can be either unilateral or bilateral, diagnosed before or after the age of one year, associated with microphthalmos, secondary glaucoma and buphtalmos. The presence of a white pupil requires correct and immediate diagnosis since, in approximately 50% of all cases, is due to retinoblastoma. Other possible causes are: advanced Coats' disease, retinal dysplasia, Norrie's disease, persistent hyperplastic primary vitreous, retinopathy of prematurity, retinoma, coloboma, retinal hamartoma, medulloepithelioma.

Conclusion In evaluating a child who presents with a white pupil, the diagnosis of retinoblastoma must be confirmed or excluded. When retinoblastoma is excluded from the differential diagnosis, the cause must be sought. Clinical history and ophthalmoscopy should be combined with CT scan, US, and MRI, and only in selected cases, with invasive techniques.

■ 2432

**Differential diagnosis of retinoblastoma:
A review of 205 children seen at Curie Institute**

DESIARDINS L (1), LEVY-GABRIEL C (1), LUMBROSO LEROUIC L (1), DOZ F (2),
 DENDALE R (3), BRISSE H (4), SASTRE X (5)

- (1) *Ophthalmology, Paris*
- (2) *Pediatric Oncology, Paris*
- (3) *Radiotherapy, Paris*
- (4) *Radiology, Paris*
- (5) *Pathology, Paris*

Purpose To determine the most frequent differential diagnosis of retinoblastoma and give clinical guidelines.

Methods Retrospective review of the charts of children sent to Curie institute for suspicion of retinoblastoma between January 2003 and December 2005. The clinical and radiological aspects of other lesions will be described.

Results During this 3 years period, 205 children were sent to Curie institute for suspicion of retinoblastoma. There were 172 retinoblastoma (84%) and 33 other diagnosis (16%). The most frequent diagnosis were coats disease (7), coloboma (5) and PHPV (3). Other diagnosis included cataract (1), hamartoma (2), medulloepithelioma (1), myelinated nerve fibers (2), retinopathy of prematurity (1), vitreous retinal dysplasia (2), microphthalmia (1), congenital iris heterochromia (2), limbus dermoid (1), infectious disease (2, one toxocara canis and one scratch eye disease) and astrocytomas (2). One child was sent for abnormal white reflex on a flash photograph and had normal fundus.

Conclusion Multiple lesions can be responsible for strabismus and leucokoria in young children. It is important to differentiate retinoblastoma from other lesions to avoid unnecessary enucleations. The possibility of diffuse infiltrative retinoblastoma should be kept in mind and sometimes make the differential diagnosis very difficult.

■ 2433

Differential diagnosis of leukocoria: Emblematic cases

MUNIER F, BALMER A, LIFFERS

Purpose The list of differential diagnosis of leucocoria is quite large and the pitfalls are numerous. Based on a series of emblematic cases, the most important clinical features and imaging characteristics will be detailed and discussed. In addition several examples of clinico-pathologic correlations will be presented.

■ 2434

The role of diagnostic imaging (MRI) in children with leucocoria

IMHOFS, MOLL A, DE GRAAF P
VU Medical Centre, Dept. Ophthalmology and Radiology, Amsterdam

Purpose The diagnosis of intraocular tumors and simulating lesions are primarily made by means of clinical examination. However MR imaging is very helpful to confirm diagnoses, to determine the extent of the intraocular tumor, and to exclude extraocular involvement. They are also valuable in differentiating retinoblastoma from simulating lesions.

Methods Pre- and post contrast MR from children with retinoblastoma and simulating intraocular lesions will be discussed.

Conclusion Pre- and post contrast MR studies allow differentiation of solid tumors such as retinoblastoma and medulloepithelioma from intraocular lesions with primary retinal detachment such as Coats disease, PHPV and congenital retinal dystrophy. MR appearance of retinoblastoma using a surface coil are compared with histopathological results.

■ 2435

Leukocoria associated with glaucoma

FREZZOTTI P (1), DE FRANCESCO S (2), MENICACCIF (3),
HADJISTILIANOU T (4)

(1) *Ophthalmology and Neurosurgery, Siena*

(2) *Ophthalmology- Ocular Oncology Unit, Siena*

(3) *Ophthalmology, Siena*

(4) *Ophthalmology - Ocular Oncology Unit, Siena*

Purpose Intraocular tumors and pseudotumors can lead to glaucoma. The most common tumors include uveal melanoma, uveal metastasis and retinoblastoma. Tumor-induced glaucomas are almost always unilateral. The mechanism of the glaucoma can vary with the size and extent of the tumor. Large, posteriorly located neoplasm can produce anterior displacement of the lens-iris diaphragm and angle closure. Such tumors also may induce iris and angle neovascularization, or they may liberate tumor cells into the anterior chamber, blocking aqueous outflow by directly infiltrating the trabecular meshwork or by liberating tumor cells that can diffusely obstruct aqueous outflow. The Authors present cases of secondary glaucoma in retinoblastoma, retinal dysplasia, PHPV, medulloepithelioma and advanced Coats disease and discuss the clinical course and mechanisms at the base of the secondary glaucoma.

■ 2441**Macular protection versus scotopic vision with blue light filtering IOLs**

SÖDERBERG PG (1, 2, 3)

(1) St. Erik's Eye Hospital, Karolinska Institutet, Stockholm

(2) College of Engineering, University of Miami, Miami

(3) Dept. of Ophthalmology, University of Dalian, Dalian

Purpose To discuss current concepts for macular protection with IOLs.**Methods** The theoretical background for macular protection with IOLs and the options available were reviewed. The consequence on vision was analyzed theoretically.**Results** The luminous efficiency function for scotopic and photopic vision is centered around 555 and 505 nm, respectively. Light energy absorbed in the oxygen rich retina, easily is transferred to oxygen. Oxygen secondarily may cause damage through free radical reactions. Exposures of the retina to light for over 12 hrs produces damage with an action spectrum that very closely resembles the scotopic luminous efficiency function and the absorption spectrum for rhodopsin. Short exposures of light produces damage with an action spectrum for the phacic eye that is centered around 440 nm. The blue light transmittance in the crystalline lens decreases with increasing age. It is believed that this is part of a natural protection of the aging retina against blue light high energy photons. This observation has stimulated IOL manufacturers to simulate the blue light absorption. First out was HOYA that more than ten years ago developed a blue light filtering PMMA IOL. A few years ago, ALCON launched their blue light filtering acrylic IOL and recently HOYA has introduced a compound acrylic optic PMMA haptic IOL. A calculation of the dampening of scotopic vision with one of the current blue light filtering IOLs shows that scotopic vision decreases less than what occurs in a normal 50 year old lens.**Conclusion** Filtering out blue light in IOLs, theoretically renders protection of the aging retina with little impact on scotopic vision. Only clinical longterm follow up studies can answer if it works.**■ 2442****IOLs correcting spherical aberrations: benefit and limitations**

ARTAL P, TABERNERO J

Lab. Optica, Universidad de Murcia, Murcia

ABSTRACT NOT PROVIDED**■ 2443****Global characterization of optical quality in eyes with various types of monofocal lenses**

MARCOS S

Instituto de Optica, Consejo Superior de Investigaciones Cientificas, Madrid

Purpose To understand contributions of different components (corneal incision, lens geometry, lens tilt and decentration) to overall ocular aberrations of eyes implanted with different types of monofocal intraocular lenses.**Methods** Our laboratory has set up several new instruments to measure the optical and geometrical properties of eyes implanted with intraocular lenses. Corneal aberrations were measured with corneal topography and custom-developed algorithms. Total aberrations were measured using a custom-developed Laser Ray Tracing system. Ocular biometry was measured using low coherence interferometry. Intraocular tilt and decentration was measured using a custom-developed Purkinje imaging system (as well as Scheimpflug imaging with custom-developed algorithms).**Results** We will review recent results obtained in our lab: 1) Optical, Corneal and Internal aberrations measured *in vivo* in eyes with aspheric and spherical IOLs. 2) Experimental estimates of optical depth-of-focus in eyes with spherical and aspheric IOLs. 3) Comparison of corneal aberrations induced by implantation of two types of aspheric IOLs (Tecnis and Acrysof IQ). 4) Comparison of optical aberrations and IOL lens tilt and decentration in eyes with those lenses. 5) Study of the contribution of individual factors to final optical quality, using custom eye models.**Conclusion** Experimental measurements using total and corneal aberrometry, as well as new tools to assess IOL positioning allow a better understanding of the relative contribution of different factors affecting global optical quality of the eye. These tools allow to identify effective differences across lens types, and potential improvements in cataract surgery.**■ 2444****The Sarfarazi Elliptical Accommodating Intraocular Lens**

SARFARAZI M

Harvard Medical School, Boston

ABSTRACT NOT PROVIDED

■ 2445

New accomodative IOL

ALIO JL
-, Alicante

ABSTRACT NOT PROVIDED

■ 2446

Current status of Phaco Ersatz

BARRAQUER RI, MICHAEL R
Barraquer Institute, Barcelona

Purpose To review the current status of research seeking the restoration of accommodation in presbyopic and cataract patients by lens refilling surgery.

Methods The Phaco-Ersatz concept involves research in 4 different areas: surgical techniques, material science, prevention of posterior capsule opacification (PCO), and accommodation physiology (AP). This presentation will review the personal experience of the authors and the published data from other groups working in these areas.

Results Extraction of the lens contents through a sub-1 mm mini-rhexis has been shown feasible in both animals and young to middle-aged humans. Current progress involves the extension of this to the harder cataracts using the newer cold ultrasound and non-ultrasound technologies. Refilling materials based on silicon polymers have been shown to have the adequate biocompatibility and optical properties to restore accommodation in monkeys. Current efforts involve the development of safer polymers that can be cured *in situ* after lens refilling and avoiding the need of UV light. PCO prevention remains a major hurdle, although new capsular plug devices show promising results regarding a safe application of agents of known efficacy but until now low risk/benefit ratio. Research in AP is required not only to understand how it is lost and can be restored, but for practical purposes as deciding the precise amount of refilling required. Ex vivo lens stretching experiments are supplying useful data regarding this aspect.

Conclusion Phaco-Ersatz remains a long-term project that still requires further efforts in multiple disciplines. The recent progresses in several areas confirm it is a viable idea whose applicability to clinical practice can already be foreseen.

■ 2451

A new approach for mass glaucoma screening

HOLLÓ G (1), TÓTH M (1), KÓTHY P (1), VARGHA P (2)

(1) Department of Ophthalmology, Semmelweis University, Budapest

(2) Biometry Unit, Semmelweis University, Budapest

Purpose To investigate the advantage of combined use of scanning laser polarimetry with customized cornea compensation (GDx-VCC) and Matrix Frequency Doubling Technology (M-FDT) in glaucoma screening.

Methods Two hundred-thirty-three Caucasian subjects were screened for glaucoma with GDx-VCC, M-FDT and independent clinical examination. The positive cases underwent a detailed clinical investigation to verify or exclude glaucoma.

Results Of the 233 participants 181 persons (345 eyes) participated in the GDx-VCC and M-FDT measurements. Thirty-nine eyes of 24 participants had glaucoma (11.3% prevalence). All but two glaucomatous eyes had early damage. When the tests were evaluated separately, the GDx-VCC NFI (normal≤30) performed best with 25.6% sensitivity, 97% specificity, 8.5 positive likelihood ratio and 89% accuracy. The best-performing combinations of GDx-VCC screening and M-FDT screening, and NFI and GDx-VCC nerve fiber bundle defect provided 28.6 and 17.9 positive likelihood ratio, 99.6% and 99.0 % specificity and 91% and 90% accuracy, respectively, but the sensitivity decreased to 12.0% and 17.9%, respectively. When the latter three criteria were combined the sensitivity increased to 41.7% and the positive likelihood ration still remained clinically significant (13.6).

Conclusion In self-reported Caucasian populations with relatively high risk for mild glaucomatous damage a combination of GDx-VCC with the M-FDT can be successfully used for mass glaucoma screening.

■ 2452

Eating Fruits and Vegetables May Decrease Risk of Glauoma

COLEMAN AL (1), TOPOULIS F (2), BADALA F (3), YUF (1)

(1) Jules Stein Eye Institute, Los Angeles

(2) Aristotle University, Thessaloniki

(3) Wills Eye Institute, Philadelphia

Purpose To explore whether the consumption of fruits and vegetables is associated with glaucomatous optic nerve damage in a cohort of older women.

Methods A random sample of 1274 older women in a population-based cohort had optic nerve photographs and suprathreshold visual field testing in both eyes. Two glaucoma specialists, who were masked to the findings of the other and to all clinical data except for the optic nerve photographs and visual field results, determined whether the women had glaucomatous optic nerve damage or not. A third glaucoma specialist adjudicated any discrepancies between their classifications. 1196 (94%) of the women completed the Block Food Frequency Questionnaire. Frequencies were compared with the Fisher's exact test, the trend of associations between fruit and vegetable consumption and glaucoma were compared with Cochran-Armitage trend test, and logistic regression modeling was performed, controlling for study sites, age, race, smoking status, and diabetes.

Results The average age in this cohort of women was 79 years. 106 (8.3%) women were diagnosed with glaucoma. Eating carrots or collard greens/kale was associated with decreased odds of having glaucomatous optic nerve damage (OR = 0.34 and OR = 0.28, respectively). Women who ate canned or dried peaches were less likely to have glaucomatous optic nerve damage (OR = 0.56), while women who drank orange juice were more likely to have glaucomatous optic nerve damage (OR = 1.95).

Conclusion Overall, the consumption of fruits and vegetables holds promise for protection against glaucomatous optic nerve damage. The increased risk associated with drinking orange juice needs to be explored further in this cohort.

■ 2453

Systemic antihypertensive medication and the risk of incident glaucoma

MUSKENS RPHM (1, 2), DE VOOGDS (2), WOLFS RCW (2, 3)

WITTEMAN JC (2), HOFMAN A (2), DE JONG PTVM (2, 4, 5), STRICKER BHC (2), JANSONIUS NM (1, 2)

(1) University Medical Center Groningen, University of Groningen, Groningen

(2) Department of Epidemiology & Biostatistics, Erasmus Medical Center, Rotterdam

(3) Department of Ophthalmology, Erasmus Medical Center, Rotterdam

(4) Department of Ophthalmology, Academic Medical Center, Amsterdam

(5) The Netherlands Ophthalmic Research Institute, KNAW, Amsterdam

Purpose To determine the association between systemic antihypertensive medication and incident glaucoma.

Methods The study population consisted of a subset of 3842 participants of the Rotterdam Study, for whom data from ophthalmologic examination at baseline and follow-up were available. Use of antihypertensive medication was registered over a follow-up period of 6.5 years. Associations between incident glaucoma and antihypertensive medication were assessed using multivariate logistic regression models adjusted for age, gender, duration of follow-up, intraocular pressure (IOP), IOP lowering medication and cardiovascular disease.

Results During follow-up, there were 87 incident cases of glaucoma. Participants using calcium channel antagonists had a 1.9 fold (95%CI 1.1-3.3) higher risk of developing incident glaucoma. None of the other classes of antihypertensives showed a significant association.

Conclusion Our data suggest that calcium channel blockers may induce glaucoma but require confirmation. Meanwhile, doctors should be aware of this potential association when treating patients with glaucoma. The use of calcium channel antagonists for normal tension glaucoma cannot be supported based on this study.

■ 2454

Frequency of Optic Disc Hemorrhages in Adult Chinese in Rural and Urban China. The Beijing Eye Study

JONAS JB (1), WANG Y (2), XU L (2), HU L (3), WANG Y (2), YANG H (2)

(1) Department of Ophthalmology Mannheim, University of Heidelberg, Mannheim, Germany, Mannheim

(2) Beijing Institute of Ophthalmology, Beijing Tongren Hospital and Capital University of Medical Science, Beijing, China, Beijing

(3) Department of Ophthalmology, The 306 Hospital of PLA, Beijing, China, Beijing

Purpose To determine the frequency of optic disc hemorrhages in the adult Chinese population

Methods The population-based prevalence survey included 4439 subjects out of 5324 subjects invited to participate (response rate 83.4%) with an age of 40+ years. Mean age was 56.2 ± 10.6 years. Color optic disc photographs (45°) were morphometrically examined.

Results Optic disc photographs were available for 8655 eyes of 4378 / 4439 (98.6%) subjects. Prevalence of disc hemorrhages was 107 / 8655 (1.24%; 95% confidence interval (CI): 1.00%, 1.47%) eyes. Occurrence of disc hemorrhages was significantly associated with glaucomatous optic nerve damage ($p<0.001$; OR: 9.3; 95%CI: 5.6, 15.4) and age ($p=0.008$; 95%CI: 1.01, 1.05). It was not significantly associated with intraocular pressure ($p=0.63$; 95%CI: 0.97, 1.06), refractive error ($p=0.06$; 5% CI: 0.87, 2.18), and visual field score ($p=0.81$; 95%CI: 0.96, 1.03). Defining glaucoma as glaucomatous optic disc appearance, 20 / 107 (18.7%; 95%CI: 11.2%, 26.2%) disc hemorrhages were found in glaucomatous eyes. Out of 226 glaucomatous eyes, 20 / 226 (8.8%; 95%CI: 5.12%, 12.58%) eyes showed a disc hemorrhage. Hypertensive glaucoma eyes and normotensive glaucoma eyes did not vary significantly in frequency of disc hemorrhages ($P=0.44$; OR: 1.82; 95%CI: 0.59, 5.68).

Conclusion Disc hemorrhages occur in a frequency of about 1.2% in adult Chinese. Major associated factors are glaucomatous optic neuropathy and age. Presence of a disc hemorrhage suggested glaucomatous optic nerve damage with a positive predictive value of about 20%. About 9% of glaucomatous eyes showed a disc hemorrhage at the time of examination.

■ 2455

The impact of dorzolamide/timolol unfixed combination on visual field in primary open-angle glaucoma patients.

MARTINEZ-GARCIA A, SANCHEZ-SALORIO M
INGO, Santiago de Compostela

Purpose To evaluate the effect of Dorzolamide 2%, twice daily (BID), on the progression of visual field damage in open – angle glaucoma patients.

Methods 80 eyes of 40 patients with asymmetric glaucomatous damage were included in a prospective open-label study. Prior to the baseline visit, patients were treated with timolol maleate 0.5%, twice daily, in both eyes for 18 months. At the baseline visit, based on the visual field result dorzolamide 2% BID was added in the eye with the most advance disease. Kaplan – Meier survival curve was used to determine the success of Dorzolamide 2% due to variable follow-up.

Results Kaplan – Meier survival analysis showed a cumulative success of 82.5% at 48 months in the eyes treated with dorzolamide 2%. In the fellow eye the cumulative survival rate was 60% at 48 months, p= 0.035. Dorzolamide 2% reduced intraocular pressure (IOP) from 19.18 (1.34) to 18.07 (1.31) mm Hg, p= 0.0001, with a mean follow-up of 45.30 (7.44) months.

Conclusion Dorzolamide 2% BID added to timolol maleate 0.5% BID seems to be an effective treatment to delay the visual field progression in patients with open-angle glaucoma.

■ 2456 / 412

Community optometrist referrals for glaucoma- referral accuracy and outcomes

MALIK ANJ, YUEN L, ALTHAUSER S
Royal Free Hospitals NHS Trust, London

Purpose The UK government commissioned National Eye Care Services report recommends changes to allow direct referrals by optometrists to hospital services, as well as new roles in community care of glaucoma patients. We aimed to determine optometrist referral patterns and the outcomes.

Methods Retrospective study of new optometrist referrals to a specialist glaucoma clinic over a six month period

Results The positive predictive value (PPV) of a positive outcome (diagnosis with glaucoma or as suspected glaucoma) was 0.35, based on their first visit (total number patients 102). Referral based on one suspicious finding occurred in 67% of patients (n=68). The most common reason stated for referral was raised intraocular pressure (IOP) in 60% of cases. The mean IOP for referral was 24mm Hg (range 13-35). In comparison the mean IOP of this subgroup was 17mm Hg (range 12-28) when measured in hospital (p<0.001). Non contact tonometry was used by optometrists in 96% of patients. Other causes for referral included suspect looking optic disc (44%) and visual field loss (22%). Visual field defects were found in 40% (23/57) of patients by optometrists, when performed. In patients referred with visual field defects 39% (9/23) had the defect confirmed at the hospital visit. The mean cup disc ratio was comparable between optometrists and the hospital glaucoma service (p=0.09). 24% of referrals documented a family history.

Conclusion The PPV of referral accuracy improves when more than one suspicious finding is documented. Non- contact tonometry and perimetry results from a single patient visit is a major source of unnecessary referrals. Emphasis on specific training and equipment of community optometrists will be required as their role in management of glaucoma increases.

■ 2457 / 413

Development of an Instrument to Assess Behavior and Readiness for Behavior Change in Patients Taking Ocular Hypotensive Therapy

SCHWARTZ GF (1), PLAKE K (2), MYCHASKIW MA (3)

(1) Glaucoma Consultants, Wilmer Eye Institute, Johns Hopkins University, Baltimore

(2) Purdue University School of Pharmacy and Pharmaceutical Sciences, West Lafayette

(3) Pfizer Inc, New York

Purpose To develop and conduct preliminary validation of a tool to assess medication-taking behavior in patients taking ocular hypotensive therapy and readiness for behavior change using the Transtheoretical Model.

Methods A survey was developed with questions derived from a review of the ophthalmic and non-ophthalmic literature and modified to apply to glaucoma or ocular hypertensive patients. The questionnaire included patient evaluations of health and medications, difficulties in taking ophthalmic medications, use of medications, visual function (NEI-VFQ 25), and demographics. The draft was reviewed for content and face validity by a panel of 8 glaucoma specialists and behavioral and health economics experts.

Results The panel confirmed the content and face validity of the questionnaire. The panel recommended reducing forced choices by adding more coded responses for selected items and amending wording to improve readability and response clarity. Additional testing in the target population is planned prior to use in patient care settings.

Conclusion The expert panel provided preliminary validation of the instrument. Application in patient care settings may allow physicians to identify medication-taking behavior and patients in whom adherence or persistence needs to be improved. Identifying patients' behaviors and issues in taking medications will allow appropriate interventions to be designed.

■ 2461

Mechanism of Action of Anecortave Acetate

DE SMET M (1, 2)

(1) Division of Vitreo-retinal and Uveitis, Department of Ophthalmology, ZNA Middelheim, Antwerp

(2) Department of Ophthalmology, Academic Medical Center, University of Amsterdam, Amsterdam

Purpose To review the mechanism of action of the anti-angiogenic cortisene, anecortave acetate.

Methods Anecortave acetate is a structural analog of cortisol that has been specifically and irreversibly modified to enhance angiostatic activity without the glucocorticoid receptor-mediated steroid side-effects (e.g., cataract and glaucoma) typically associated with a steroid. Fourteen *in vitro* and *in vivo* models of ocular angiogenesis have been used to elucidate its mechanism of action.

Results The angiostatic activity of anecortave acetate is attributed to actions upstream of vascular endothelial cell (VEC) activation and downstream of proangiogenic factor activation. The latter is a result of blockade of the proteolytic cascade by upregulation of urokinase plasminogen activator and down regulation of the expression of urokinase plasminogen activator and pro-matrix metalloproteinases (proMMPs). This results in the suppression of the matrix metalloproteinases necessary for migration and proliferation of activated VECs. Additional data from cell culture experiments show independent actions targeting proliferation and tube formation. Anecortave acetate also acts upstream of the activation of proangiogenic factors by inhibiting the early onset of over expression of VEGF and delayed onset of IGF-1 expression in an animal model of preretinal neovascularization.

Conclusion Anecortave acetate acts at multiple sites to block the angiogenesis cascade and prevent neovascularization independent of the inciting stimulus. The consequence of this unique mechanism of action means that combinations of anti-angiogenic therapies with different mechanisms of action may be used. Investigations of these combinations are now being conducted for treatment of exudative age-related macular degeneration.

■ 2462

Photodynamic therapy associated to peri-bulbar triamcinolone injection in the treatment of subfoveal choroidal neovascularization

MAESTRONI L, BARTESELLI G, MIGLIOR S

Clinica Oculistica, Policlinico di Monza, Università Milano Bicocca, Monza

Purpose To asses the efficacy and safety of peribulbar injection of triamcinolone combined to PDT in subfoveal choroidal neovascularization (CNV) secondary to ARMD.

Methods 25 patients affected by subfoveal CNV secondary to ARMD, eligible for PDT, were treated with PDT combined with peribulbar injection of triamcinolone. All these patients were not previously treated. At each visit ETDRS visual acuity, IOP and biomicroscopic evaluation of anterior and posterior segment, digital dynamic fluorescein angiography, digital dynamic indocyanine green angiography by means of HRA, and OCT were performed. Follow up visits were planned quarterly for 1 year. In case of re-treatment that could have been necessary after three months from previous dosing, the same combined procedure was administered.

Results The follow up ranged between 12 and 14 months. Combined therapy resulted safe. There was no ocular and systemic adverse event including any rise of intraocular pressure or infection. In two cases a mild ptosis that not required any treatment was observed. Mean visual acuity at baseline was 20/100 (range 20/200-20/80), and at the last follow up visit was 20/100 (range 20/200-20/50). The re-treatment rate was 2.9. Retinal thickness measured by OCT showed an average decrease of 17% (SD 11%).

Conclusion In this pilot study, the combined treatment appears to be safe, and seems capable to maintain a stable visual acuity over a period of 12 months in patients with subfoveal CNV secondary to ARMD. It seems also capable of reducing re-treatment rate when comparing our figures with previously published re-treatment rates (about 3.4). These preliminary data need to be confirmed by a randomized controlled clinical trial.

■ 2463

Pegaptanib Sodium in the Treatment of Patients with Macular Oedema Following Central Retinal Vein Occlusion (CRVO)

PATEL S, MACUGEN RETINAL VEIN OCCLUSION STUDY GROUP (OSI) Eyetech, Inc., New York

Purpose Selective VEGF-A inhibition has shown positive outcomes in the treatment of diabetic macular edema. Levels of VEGF, a key promoter of angiogenesis and vascular permeability, also increase in response to hypoxia induced by retinal vein occlusion. This study evaluated the safety and potential benefit of pegaptanib sodium in treating macular edema secondary to CRVO.

Methods This was a randomized, double-masked, sham-controlled, multicenter trial that enrolled subjects with recent vision loss resulting from macular edema secondary to CRVO. Every 6 weeks for 24 weeks subjects received either intravitreous pegaptanib sodium (0.3 mg or 1 mg) or sham injections. In accordance with the Central Vein Occlusion Study protocol, photocoagulation for neovascularization was performed if required. Endpoints included visual acuity and optical coherence tomography, fluorescein angiography, and color photography results.

Results Ninety eight subjects were included (47 women, 51 men; baseline mean age, 61.8 years; baseline mean visual acuity in study eye, 50.4 letters [\sim 20/100 Snellen equivalent]). Preliminary first year data and ancillary testing results will be presented along with representative individual cases.

Conclusion Selective VEGF inhibition may have a significant role in the treatment of ischemic retinal diseases including CRVO.

■ 2464

Benefits of Pegaptanib Sodium in Subjects with Diabetic Macular Oedema

CLINHA-VAZ J (1, 2, 3), FOR THE MACUGEN DIABETIC RETINOPATHY STUDY GROUP (4)

(1) Ophthalmology Department of the University Hospital, Coimbra

(2) Centre of Ophthalmology of University of Coimbra, Coimbra

(3) AIBILI, Coimbra

(4) Eyetech, New York

Purpose Studies indicate that VEGF plays a key role in the pathogenesis of diabetic macular edema (DME). A phase 2 clinical trial was conducted to evaluate the safety and efficacy of selective VEGF blockade with pegaptanib sodium in subjects with DME.

Methods In a double-masked, randomized, controlled trial involving 172 subjects with DME, pegaptanib sodium (0.3 mg, 1 mg, or 3 mg) or sham injections were administered at baseline, weeks 6 and 12. Additional injections and/or focal photocoagulation were administered at the investigator's discretion for 18 additional weeks, with endpoint assessments at 36 weeks and final assessments at 82 weeks. The endpoints included visual acuity, retinal thickness, and need for additional photocoagulation. A retrospective analysis examined the effects of pegaptanib on retinal neovascularization.

Results Pegaptanib sodium was superior to sham for all endpoints. Mean change in visual acuity was +4.7 vs. -0.4 letters for the 0.3 mg dose vs. sham ($P=0.04$). More patients receiving pegaptanib maintained or gained ≥ 5 , 10, or 15 letters and fewer subjects receiving the 0.3 mg dose than those receiving sham required laser. Of 16 subjects with retinal neovascularization in the study eye, 8/13 treated with pegaptanib and 0/3 receiving sham, as well as 0/4 in the non-study eye, had neovascular regression at week 36. One year after stopping pegaptanib therapy the mean change in visual acuity was +1.3 vs. -2.4 letters for the 0.3 mg dose vs. sham.

Conclusion Pegaptanib sodium resulted in improvements in mean visual acuity, decreased central retinal thickness, regression of neovascular lesions, decreased disease severity and decreased likelihood of requiring laser therapy in subjects with DME.

■ 2465

Symmetry of age related maculopathy and macular degeneration in the London AMD Study Cohort

PETO T (1), DANDEKAR SS (1), RUTISHAUSER-ARNOLD Y (2), JENKINS S (1)

WEBSTER AR (3), LEILING I (1), BIRD AC (3)

(1) Moorfields Eye Hospital, London

(2) Stadspital Triemli, Zurich

(3) Institute of Ophthalmology, London

Purpose To determine the degree of concordance in eyes of patients with age-related maculopathy (ARM) and late stage age-related macular degeneration (AMD).

Methods The London AMD Study was a prospective, observational, cross-sectional study of 1468 patients. Of these, 162 had bilateral drusen and 375 had bilateral AMD verified by grading colour fundus photographs and fluorescein angiograms based on a modified version of the International Classification. For ARM, all drusen characteristics (size, type, number, area covered) and pigmentary changes (hypo- and hyperpigmentation) were recorded. For AMD, characteristics and concordance of geographic atrophy (GA), choroidal neovascularisation (CNV) and pigment epithelial detachment (PED) were noted. Kappa statistics were used to determine concordance greater than that due to chance alone.

Results In ARM, drusen and pigmentary change characteristics were highly concordant, with kappa values all in the moderate to substantial range with a high degree of significance (drusen: 57-90%, kappa = 26-66%, p<0.05; pigmentary changes: 64-96%, kappa = 0.22 - 38, p<0.05). In late stage AMD, concordance of phenotype was observed in 281/375 (75%) patients (kappa= 0.48, p<0.001); 82 patients had GA, 5 had PED and 194 had CNV in both eyes. The concordance rate for neovascular AMD (CNV+ PED combined) was 77% (kappa= 0.49, p<0.001).

Conclusion The observed high concordance of ARM confirms previous studies. However, discordance for the type of lesion in AMD was seen in 25%. This suggests that genetic and environmental factors common to both eyes determine the characteristics of early ARM, but that the final lesion in late AMD has a stochastic component.

■ 2466

Visual Acuity Change after Intravitreal Bevacizumab for Exudative Age-Related Macular Degeneration in Relation to Subfoveal Membrane Type

LIBONDI T (1), IHLOFF AK (2), HARDER B (2), KREISSIG I (2)

SCHLICHTENBREDE F (2), SPANDAU UHM (2), JONAS JB (2)

(1) Dept of Ophthalmol. II Univ Napoli, Napoli

(2) Dept of Ophthalmology, Faculty of Clinical Medicine Mannheim of the University Heidelberg, Mannheim

Purpose To evaluate the change in visual acuity after an intravitreal injection of bevacizumab for treatment of exudative age-related macular degeneration (AMD) with respect to the type of subfoveal neovascular membrane.

Methods The clinical interventional case-series study included 44 patients (44 eyes) with exudative AMD who received an intravitreal injection of 1.5 mg bevacizumab. According to the appearance of the subfoveal neovascular membrane in fluorescein angiograms, the whole study group was divided into a occult type (n=18 (47%) eyes), minimally classic type (n=5 (13%) eyes), predominantly classic type (n=12 (32%) eyes), and purely classic type (n=3 (8%) eyes). Follow-up was at least 2 months.

Results The maximal gain in visual acuity (mean ± S.D.: 2.8 ± 3.1 Snellen lines), and the gain in visual acuity at 1 month (1.8 ± 3.1 Snellen lines) and 2 months (2.4 ± 3.4 Snellen lines) after the injection did not vary significantly between the four study subgroups (P=0.14; Analysis of variance). Correspondingly, a multivariate analysis with age, type of subfoveal lesion and baseline visual acuity as independent variables did not reveal a statistically significant (P=0.71) influence of subfoveal lesion type on gain in visual acuity.

Conclusion The gain in visual acuity after an intravitreal injection of bevacizumab for treatment of exudative AMD does not markedly differ between the various types of subfoveal neovascularization.

■ 2467 / 301

Short term effect of intravitreal Bevacizumab (Avastin) for exudative age-related macular degeneration

CHRISTMANN S (1), CORDES AK (1), DOHM S (1), SCHRAGE NF (1), DEGENRING RF (1, 2)

(1) Dept. of Ophthalmology Cologne-Merheim, Cologne Hospitals, Cologne

(2) Faculty of Clinical Medicine Mannheim, University of Heidelberg, Mannheim

Purpose To evaluate the clinical effect of intravitreal bevacizumab injections in exudative age related macular degeneration (AMD).

Methods Retrospective study. 16 eyes (16 patients) suffering from exudative AMD of all types were treated by single or repeated intravitreal injections of 1 mg Bevacizumab (Avastin). Mean number of injections was 2.3 +/- 0.9 (range, 1-3 injections; median, 3 injections) in intervals of 4 weeks. Fluorescein angiography (FA) was performed preoperatively and regularly during follow-up. Available pre- and postoperative FAs were mixed and then qualitatively compared by a masked person. Mean patient age was 73.9 +/- 8.7 years (range, 55-88 years).

Results Visual acuity (VA) increased significantly (p=0.032; t-test for paired samples) from preoperatively 0.16 +/- 0.36 (range, 0.03 to 0.8; median, 0.2) to 0.20 +/- 0.39 (range, 0.05 to 0.6; median, 0.3) at the end of follow-up. In 8 eyes (50%) VA improved at least one line at the end of follow up, in 5 eyes (31%) VA was stable, and in 3 eyes (19%) VA deteriorated one or two lines. In average, VA improved 1.1 +/- 2.1 lines (range, -2 lines to +5 lines; median, 0.5 lines). In 12 of 16 FAs (75%) the masked examiner judged the postoperative angiogram to be „better“, in 3 FAs (19%) the postoperative FA was graded to be „worse“, and in 1 case (6%) the examiner was „undecided“. No ocular adverse events like inflammation, retinal detachment or increase of the intraocular pressure were observed.

Conclusion In this retrospective study of single or repeated intravitreal bevacizumab injections in exudative AMD we found significant improvement of visual acuity and qualitative improvement in fluorescein angiography during short term follow-up.

■ 3111

The Role RPE in Aging Eye

UUSITALO H

*Dept of Ophthalmology, University of Kuopio, Kuopio***ABSTRACT NOT PROVIDED**

Purpose Lipofuscin accumulation in the RPE is a common downstream pathogenic pathway in various monogenic and complex retinal diseases including age-related macular degeneration (AMD). We tested the hypothesis that lipofuscin-associated compounds may induce lysosomal dysfunction of RPE cells.

Methods The retinoid derivative A2-E, the lipid peroxidation-derived aldehydes malondialdehyde (MDA) and 4-hydroxynonenal (HNE), and POS proteins modified by lipid peroxidation products were analysed for their adverse effects on lysosomal function in cultured RPE cells and in isolated lysosomes.

Results A2-E was shown to induce adverse alterations of the lysosomal milieu by inhibiting the lysosomal proton pump, to cause leakage of the lysosomal membrane and to act as a photosensitizer. HNE and MDA are capable of inactivating lysosomal proteases by forming Michael adducts with amino acids located at the active center of the enzymes. Furthermore adduct formation of the aldehydes with phagocytosed proteins causes their stabilization towards lysosomal degradation thereby inducing their intralysosomal storage. Proteins carbonylated by lipid peroxidation products are also effective as proteolytic antagonists thereby acting as general lysosomal proteolytic inhibitors.

Conclusion Our results indicate that A2-E and lipid peroxidation-derived aldehydes are potent inducers of lysosomal dysfunction and intalysosomal storage in RPE cells. Thus these compounds are likely to be involved in lipofuscinogenesis and may contribute to cell damaging effects of lipofuscin in retinal diseases such as AMD.

■ 3113

Proteasomes and Heat shock Proteins in Protein aggregation of RPE cells

KAARNIRANTA K

Dept of Ophthalmology, University of Kuopio, Kuopio

Purpose Once heat shock protein (Hsp) mediated protein folding fails, the misfolded proteins are tagged with a ubiquitin prior to proteasomal degradation. Protein aggregates formed at the cell periphery are delivered along microtubulus network by retrograde trafficking to a juxtanuclear location. The tubulin undergoes many post-translational modifications, e.g. acetylation what is related to regulation of microtubule stability. Role of Hsp70, Hsp90, proteasome inhibition and microtubulus acetylation were investigated in cellular aggregation in human RPE cells (ARPE-19).

Methods Accumulation of Hsp90, Hsp70, Hsc70, acetylated tubulin and ubiquitinated proteins were analyzed by Western blotting. Protein aggregation was studied by immunofluorescence analysis and transmission electron microscopy. Small interference RNA (siRNA) technique was used to inhibit hsp70 mRNA.

Results Proteasome inhibition causes robust accumulation of Hsp70 protein and ubiquitinated protein conjugates in ARPE-19 cells. Hsp90 inhibitor geldanamycin resulted in a decreased protein aggregation, when proteasomes function was prevented. The amount of protein aggregates were clearly increased in response to hsp70 siRNA. Deacetylase inhibitor trichostatin and tubulin stabilizer taxol clearly increased tubulin acetylation and decreased amount of juxtanuclear protein aggregation. Nocodazole, which destabilizes microtubules had no clear effects on tubulin acetylation or proteasome inhibitor-induced aggregation.

Conclusion This study reveals that ubiquitin–proteasome pathway is an important way to control protein turnover in the RPE cells. In addition, Hsp90 and Hsp70 and tubulin acetylation are closely related to regulation of the juxtanuclear protein aggregation.

■ 3112

Lipofuscin Accumulation and Lysosomal Dysfunction in RPE Cells

KOPITZ J (1), KROHNE T (2), BERGMANN M (1), KAEMMERER E (1), SCHUTTF (3), HOLZ FG (4)

(1) Dept of Pathology, University of Heidelberg, Heidelberg

(2) Dept of Ophthalmology, Bonn

(3) Dept of Ophthalmology, University of Heidelberg, Heidelberg

(4) Dept of Ophthalmology, University of Bonn, Bonn

Purpose Lipofuscin accumulation in the RPE is a common downstream pathogenic pathway in various monogenic and complex retinal diseases including age-related macular degeneration (AMD). We tested the hypothesis that lipofuscin-associated compounds may induce lysosomal dysfunction of RPE cells.

Methods The retinoid derivative A2-E, the lipid peroxidation-derived aldehydes malondialdehyde (MDA) and 4-hydroxynonenal (HNE), and POS proteins modified by lipid peroxidation products were analysed for their adverse effects on lysosomal function in cultured RPE cells and in isolated lysosomes.

Results A2-E was shown to induce adverse alterations of the lysosomal milieu by inhibiting the lysosomal proton pump, to cause leakage of the lysosomal membrane and to act as a photosensitizer. HNE and MDA are capable of inactivating lysosomal proteases by forming Michael adducts with amino acids located at the active center of the enzymes. Furthermore adduct formation of the aldehydes with phagocytosed proteins causes their stabilization towards lysosomal degradation thereby inducing their intralysosomal storage. Proteins carbonylated by lipid peroxidation products are also effective as proteolytic antagonists thereby acting as general lysosomal proteolytic inhibitors.

Conclusion Our results indicate that A2-E and lipid peroxidation-derived aldehydes are potent inducers of lysosomal dysfunction and intalysosomal storage in RPE cells. Thus these compounds are likely to be involved in lipofuscinogenesis and may contribute to cell damaging effects of lipofuscin in retinal diseases such as AMD.

■ 3114

Macular and peripheral gene expression differences in human eyes: significance for macular degenerations

MULLINS RF

Center for Macular Degeneration, Department of Ophthalmology and Visual Science, The University of Iowa Carver College of Medicine, Iowa City

Purpose Best disease, age-related macular degeneration and other maculopathies primarily affect a relatively small portion of the posterior pole. Other diseases primarily spare the macula from degeneration. Anatomical differences are well-appreciated between the macular and extramacular regions; biochemical and gene expression differences are less well understood, however.

Methods We performed immunohistochemical studies, Western blotting and quantitative RT-PCR on tissues derived from normal human eyes, eyes with age-related macular degeneration and eyes with Best disease. Macular and extramacular regions were evaluated and compared.

Results For some proteins analyzed, expression in the macula was significantly different than extramacular expression. In the case of intercellular adhesion molecule-1, the macular choriocapillaris exhibited higher expression than the periphery, whereas the opposite pattern was observed in the external limiting membrane. For bestrophin, the product of the VMD2 gene that is mutated in Best disease, higher expression was observed in the peripheral RPE than in the macular RPE. This finding was confirmed by Western blot and RT-PCR analysis.

Conclusion The regional patterns of gene expression in the human eye may explain the propensity of the macula to undergo inflammatory changes in AMD and may explain how bestrophin mutations lead to macula-specific lesions.

■ 3115

**The complement cascade and age-related macular degeneration,
a review**

IMMONEN I

Helsinki University Eye Clinic, Helsinki

Purpose To review current knowledge on the relation between the complement cascade and age-related macular degeneration.

Methods Current published data on the complement cascade and AMD will be reviewed.

Results The Y402H polymorphism of the complement factor H (CFH) has been recently been confirmed as the major risk factor for AMD. Also anomalies at other sites of the complement cascade may modify the risk of AMD. Based on the location of the Y402H polymorphism and data from epidemiological studies, interaction of CFH with C-reactive protein may have an important role in the pathogenesis of AMD. Correlation between the CFH polymorphisms and the phenotype of especially late AMD is still unclear.

Conclusion Identification of the role of the complement cascade in the pathogenesis of AMD may prove to be a breakthrough in our understanding of the pathogenesis of AMD. Further studies on this interaction may open possibilities for preventing the development of AMD.

■ 3121

VEGF-A Forms Part of the Adaptive Response to Ischemia Responsible for Retinal Neuroprotection

SHIMA DT (1), NISHIJIMA K (1), NG YS (1), JO N (1), AKITA J (1),
 NAKAZAWA T (1), ROBINSON GS (1), ADAMIS AP (2)
 (1) (OSI) Eyetech Research Center, Lexington
 (2) (OSI) Eyetech, Inc., New York

Purpose To assess the neuroprotective role of vascular endothelial growth factor-A (VEGF-A), an important neuroprotectant in the CNS, in the retina.

Methods An ischemic injury model was used to investigate the neuroprotective effects of exogenous and endogenous VEGF-A.

Results Intravitreously administered VEGF-A produced a dose-related decrease in retinal neuronal cell apoptosis 24 hours after ischemic injury. VEGF120 and 164 showed equal potency, but VEGF164 induced significant retinal edema. VEGF120 minimized the reduction in retinal thickness associated with progressive neuronal loss 2 weeks postischemia with no major increase in edema. Also, VEGF-A (1) increased postischemic retinal blood flow and a nitric oxide synthase inhibitor reduced VEGF-A's neuroprotective effect by about 50% and (2) reduced neural cell apoptosis in an ex-vivo model in absence of blood vessels and blood flow suggesting a direct VEGF-A protective effect. VEGFR2 expression also was found in retinal neuronal cell layers and an agonist of VEGFR2 but not VEGFR1 provided neuroprotection. VEGF-A played a role in the adaptive response to retinal ischemia. Ischemic preconditioning prior to ischemia-reperfusion injury increased levels of VEGF-A and decreased apoptosis. The protective effect of preconditioning was reversed by VEGF-A inhibition with sFlt-1 or VEGF-A neutralizing antibody.

Conclusion VEGF-A appears to play a role in the endogenous adaptive response for protecting neural cells during retinal ischemia. Exploration of both vascular and neuronal cell outcomes following the use of VEGF-A antagonists to treat ischemic neovascular conditions, such as diabetic retinopathy, is needed.

■ 3122

Automated perimetry and laser polarimetry in the assessment of nerve fibers involvement in pituitary adenoma

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

Purpose To point out nerve fibers involvement in patients suffering from pituitary adenomas and submitted to transphenoidal surgical intervention.

Methods 17 patients (51+/-18 y) been selected to be submitted to a complete ophthalmic examination before and after surgery. The postintervention examinations were scheduled at 1, 3, 6, 12 months. The adenomas have been classified according to Hardy classification. A kinetic test (HFA-II, stimulus 1/4) was performed as a screening and in case of abnormal outcomes a static test 24-2 or 30-2 was carried out to better characterized the visual field (VF). The laser polarimetry of the retinal nerve fiber layer (RNFL) was performed with GDx-VCC.

Results 3 adenomas were classified as micro and 14 as macro with different dimension and extrasellar extension. No patient suffering from microadenoma had VF or RNFL damage. Among the macroadenomas, in one case reliable tests weren't obtainable, 8 subjects had no clinic mark of nerve fiber involvement, one had homonymous and GDx defects reproducible in the follow-up. 4 patients had a typical chiasmal defect before surgery and in 3 cases the VF got back to normal after surgery. The only case with permanent bitemporal defect also pointed out a reproducible RNFL thinning with GDx.

Conclusion The nerve fiber involvement can be pointed out only for macroadenomas with extrasellar extension classified as 2/C but a strict correlation between the type of clinical defect and anatomical characteristics of tumors cannot be determined. The nerve fibers involvement seems to be mainly due to compression without fiber loss as demonstrated by complete postoperative recovery of VF and normal GDx outcomes, noticed in most cases.

■ 3123

Variability of control in intermittent exotropia

HOLMES JM, MOHNEY BG, LESKE DA
Ophthalmology, Mayo Clinic College of Medicine, Rochester

Purpose Patients with intermittent exotropia (IXT) spend differing amounts of time with a manifest deviation, and the ability to hold the eyes straight has been termed "control." We have previously described an office-based control scale to categorize the severity of IXT, and we now evaluate the inter-observer test-retest reliability of the control scale.

Methods 18 patients with IXT were prospectively enrolled with angles of distance deviation ranging from 14 pd to 47 pd. Control was evaluated by two independent examiners, within 15 minutes of each other, for distance and near fixation. The scale ranges from 0 to 5, where 5 = constant exotropia, 4 = exotropia > 50% of the exam before dissociation, 3 = exotropia < 50% of the exam before dissociation, 2 = no exotropia unless dissociated, recovers in > 5 seconds, 1 = recovers in 1-5 seconds, 0 = recovers in < 1 second (phoria). Agreement between distance and near scores was evaluated by kappa statistical methods.

Results Scores ranged from 1 to 5 at distance and 0 to 5 at near. Agreement of scores between examiners was substantial at distance ($k=0.70$) but only moderate at near ($k=0.42$). 2 of 18 (11%) scores differed by more than one level for distance, and 4 of 18 (22%) scores differed by more than one level for near.

Conclusion A 6-point control scale was useful in categorizing the severity of exotropia in most patients, but the agreement between 2 examiners was not optimal. Assessing control at a single point in time may be insufficient to accurately categorize the severity of IXT in an individual patient. Further studies are underway to document control at several times during the day, and to determine whether a composite of multiple assessments better characterizes an individual patient.

■ 3124

VFA-K test: A new pediatric eye test for the general pediatric population

KOZEIS N (1, 2)
 (1) Pediatric Eye Department, Hippokrateion Hospital, Thessaloniki
 (2) 3rd University Eye department, Thessaloniki

Purpose To present a new pediatric eye test for the general pediatric population

Methods The test is consisted of a book spiral of 9 pages, a pen torch and a tennis ball. The test assesses 10 visual parameters : visual attention, far and near visual resolution and acuity, peripheral vision, color vision, contrast sensitivity, binocular status, smooth pursuits, saccadic ocular movements, eye-hand coordination, in a functional way. Each parameter is scored separately and the total score is called index K. The reliability of the test was assessed by two examiners in a sample of 80 developmentally delayed children and 80 healthy children (3months-16 years old).

Results The statistical analysis showed that: (i) the test is reliable when used by different examiners ($p=0.423>0.05$) and ($p=0.414>0.05$) (ii) the test is reliably repeatable ($p=0.104>0.05$) and ($p=0.013>0.01$), (iii) the test can reliably identify changes of the visual function ($p=0.000<0.05$) κ_{au} ($p=0.000<0.05$).

Conclusion The VFA-K test is a reliable test that could be used in any child (developmentally delayed or not). The index K could be used as an index to follow the progress of visual function in a global manner; it could also be compared with other developmental indices.

■ 3125

Complementary Medicine for blepharospasm, in patients without effect of Botulinum toxin A

NEPP J (1, 2, 3), RICHTER-MÜKSCH S (4)

(1) Ophthalmology Medical University Vienna, Vienna

(2) J Bischko Institut Acupuncture, Vienna

(3) Academy of Psychotherapeutic Medicine, Vienna

(4) Ophthalmology, Medical University, Vienna

Purpose To evaluate the effect of complementary medicine on patients suffering from primary blepharospasm, who did not respond on Botulinum Toxin A. We tried to improve visual dysfunction and quality of life with relaxing methods.

Methods Patients suffering from blepharospasm more than one year were included. We used acupuncture, and Jakobson's progressive muscle relaxation for relaxation. Sessions were performed once a week: Jakobson followed by acupuncture for 3 months, followed by Jakobson only for another 3 months. Acupuncture was performed according to the STRIKTA rules. Observations were done with subjective estimation of the severity of spasm by the visual analogue scale (VAS 10 parts), Elston Score for the handicap (1 = blindness, 6 = paraesthesia without handicap).

Results 10 patients without any effect after Botulinum Toxin were observed. The VAS improved from 8,6 mean to 3,1 after one year. The Elston score improved from 3,0 mean to 4,7. General spasticity reduced within one year. The effect of relaxation was not visible within the first session, but after about 3 months patients felt relaxed during each session. The durability of the effects was short, but increased until the last session after one year.

Conclusion The handicap of patients with Blepharospasm not responding on Botulinum toxin can be influenced with Jakobson's Relaxation in combination with acupuncture. The disadvantages of the method is the long and intensive care to influence the complaints. But there does not exist any other effective treatments, therefore the described methods may be a first approach to influence the severity and handicap of blepharospasm.

■ 3126

Edmonde Mariotte (1620-1684), pioneer of neurophysiology and neuro-ophthalmology

GRZYBOWSKI AE (1), AYDIN P (2)

(1) Department of History of Medicine, Poznań University of Medical Sciences, Poland, Poznań

(2) Mesa Hospital, Eye Department, Ankara

Purpose The aim of the study was to analyze the details and circumstances of the blind spot's discovery and analyse the scientific activity of Edme Mariotte as a physiologist and a physicist.

Methods Typical for the study in the field of the history of science.

Results The best definition ever for visual fields was given by Harry Moss Traquair (1875-1954), in his book (*An Introduction to Clinical Perimetry*, 1927) on visual fields which has become a classic: Visual fields are the seeing island in a sea of blindness. Following this description, we can say that the blind spot is a hole of blindness in the island of seeing which is actually in the great sea of blindness. The blind spot in the visual fields represents the disc of the optic nerve itself in the eye. Edme Mariotte was the first scientist who discovered it, therefore it is named after him.

Conclusion Edme Mariotte made several important discoveries in different fields of science, such as physics, mechanics, hydraulics, optics, plant physiology, meteorology, surveying, and research methodology. In medicine he is predominantly recognized as the man who discovered the blind spot, although, what is less remembered, he also extensively worked on optics and color perception. Honored as the man who introduced experimental physics into France, he played an important role in the work of the Paris Academy of Science. He falsely assumed that the site of perception in an eye was the choroid, not the retina; however, because of his experiments and interest in the eye fundus he can be treated as a pioneer of ophthalmoscopy.

■ 3127 / 401

Early diagnosis of ocular motility alterations in thyroid autoimmune ophthalmopathy (TAO)VARANO L, CARNOVALE SCALZO G, CAMPAGNA F, SCORCIA GB,
ROTONDO E, PENDINO V, PARISE S, BRUZZICHESI D, PAOLA A, SCORCIA G
Eye Clinic, Magna Graecia, University, Catanzaro

Purpose Object of our study is early diagnosis, through EOMG exam, of ocular motility alterations in subjects affected by TAO without clinical evidences.

Methods 40 eyes of 20 patients affected by TAO without clinical evidences were examined. All the patients were submitted to ocular motility exam and EOMG exam. Results were related with a group of 46 eyes of 23 subjects with same age and sex without ocular motility alterations and not affected by TAO. We used BM6000MOE system and WinEOMG software for signal stimulation and acquisition, WinElabEOMG software for results elaboration.

Results In 15% of observed patients we found an increase of movement speed dissinergy; in 60% of patients we found an increase of movement maximal speed; in 45% we found a decrease of movement medium speed.

Conclusion In TAO affected patients without evident ocular clinical signs, the analysis with EOMG sistem could bring an ocular motility alteration predictive value.

■ 3131

Pathology of radiation-induced complications

KIVELÄ T

Helsinki University Central Hospital, Department of Ophthalmology, Helsinki

ABSTRACT NOT PROVIDED

■ 3132

Biology of radiation-induced complications

DENDALE R, BOLLET M, COSSET JM

Radiation Oncology, Paris

Purpose Radiation effects are generally divided into two categories: deterministic and stochastic. Deterministic effects occur only above a certain dose level, a "threshold"; their severity then depends on the absorbed dose. Stochastic effects on the other hand have no threshold but their risk is proportional to the absorbed dose. There are two types of tissue organizations: hierarchical and flexible. Hierarchical tissues contain several compartments (stem cell, maturation, differentiated cell compartments). In flexible tissues, there are no individualized stem cells, all cells can, if necessary, play this role. The eye is made up of several tissues that can also fall into two categories: serial organs (optic nerve, ...) and parallel organs (retina, ...). The severity of a parallel organ failure depends on the percentage of its volume that has been irradiated. Serial organs on the other hand can suffer from catastrophic complications due to a lesion of a very small volume but at a very critical location. Both the protraction (total duration time) and the fractionation (dose per fraction) of radiotherapy play key roles in the development of radiation toxicities. The protraction-sensitivity depends on the cell renewal speed of the tissue, while the fractionation-sensitivity on its alpha/beta ratio in the linear quadratic model. Radiation oncologists also distinguish effects or complications according to the time of their occurrence. A generally accepted definition would call early, those occurring within 6 months after completion of the radiotherapy and late, those after. The appearance of either one or the other depends on the different tissue features named above.

■ 3133

Liverpool approach to complications of radiotherapy of uveal melanoma

DAMATO B

Ocular Oncology Service, Liverpool

Purpose To discuss the approach to the treatment of radiation-induced complications after treatment of uveal melanoma in Liverpool.

Methods An overview of the Liverpool approach to the treatment of radiation-induced complications, such as macular oedema, exudative retinal detachment and neovascular glaucoma, with presentation of illustrative case-reports.

Results The ocular oncology service in Liverpool receives around 250-300 patients with uveal melanoma each year. Most patients are treated with ruthenium-106 brachytherapy or proton beam radiotherapy. Radiation-induced complications are categorized as: (a) direct; and (b) indirect. Direct complications are optic neuropathy, maculopathy, cataract, and blepharitis. Indirect complications include: exudative maculopathy, serous retinal detachment, neovascular glaucoma, and keratopathy. Direct complications are prevented by reducing the dose of radiation to the relevant part of the eye, by eccentric plaque placement or by administering proton beam radiotherapy with a notch, if necessary through the closed eyelid to avoid direct damage to lid margin and indirect damage to cornea. Exudative maculopathy is treated by administering intraocular triamcinolone or transpupillary to the exudative tumour. Serous retinal detachment and neovascular glaucoma related to bulky residual irradiated tumour are treated by removing the ischaemic and exudative tumour, by trans-scleral resection or by endoresection.

Conclusion In Liverpool, radiation-induced complications are categorized as direct and indirect, the former often being preventable and the latter treatable by eradicating or removing the residual 'toxic tumour'.

■ 3134

Leiden approach to long-term complications of brachytherapy of uveal melanoma

JOURNÉE DE KORVER H

~, Leiden

ABSTRACT NOT PROVIDED

■ 3135

Paris approach to radiation-induced intraocular complications

DESJARDINS L (1), LEVY-GABRIEL C (1), LUMBROSO-LEROUIC L (1),

DENDALE R (1), DELACROIX S (2), PLANCHER C (3), ASSELAIN B (3)

(1) *Ophthalmology, Paris*

(2) *Radiophysics, Orsay*

(3) *Biostatistiques, Paris*

Purpose Purpose : to present an evaluation of the role of TTT combined to proton beam to prevent radiation induced ocular complications : The possible role of anticoagulant and anti aggregant therapies will also be discussed

Methods Between February 1999 and April 2003 all the patients treated for uveal melanoma by proton beam who had a tumour of 7 mm or more in thickness or 15 mm or more in diameter were included when they accepted after informed consent .Half of the patients received proton beam alone (60 grays in four fractions) and half of them received proton beam same dose followed by TTT at one month , 6 months and 12 months .All the informations concerning the initial parameters of the tumour , treatments used and follow up were registered and a statistical analysis was performed .Anticoagulant medications were used in isolated cases of radiation papillopathy and maculopathy

Results 151 patients were randomised. The median follow up is 43 months. The two groups of patients were similar in age, gender and tumour characteristics. For patients who received laser the median characteristics of each spot of laser was of 2mm spot of 500 mw for 3 mn .The patients who received laser have more tumour thickness decrease (p 0,06), less retinal detachment at the most recent follow up (p 0,14) and less secondary enucleations (p 0,02)isolated cases of retinal reapplication after TTT will be shown as well as possible cure of radiation papillopathy by anticoagulant treatment

Conclusion TTT on the tumour scar after proton beam can reduce ocular morbidity in uveal melanoma.Further studies are needed to know the best schedule for TTT and best indications.

■ 3136

Ocular complications after radiotherapy of extraocular tumour

MIDENA E

Department of Ophthalmology, University of Padova, Padova

Purpose Complications of the irradiation of intraocular tumors are well known, and represent the main cause of functional failure of conservative therapy. Notwithstanding, the main target of therapeutic irradiation is – in these cases - into the eye, and clinicians are well aware of these long term side effects. Unfortunately, anterior and posterior segment complications of therapeutic irradiation represent a relevant problem even when the eye is the "indirect" target of irradiation. Orbital and peri-orbital tumors are frequently treated with external beam irradiation, and the eye suffers from secondary side effects because it is included into the irradiation field. Sometimes both eyes are affected. Ocular long term complications of "indirect" eye irradiation vary – in incidence and severity - according to: irradiated field, radiation dose and daily rate, past medical history, and concurrent chemotherapy. The incidence of ischemia-induced complications (ischemic chorioretinopathy and neovascular glaucoma) strictly depends on the portal through which irradiation is administered. Anterior segment complications may be the cause of blindness, and sometimes enucleation. A better knowledge (incidence, risk factors, natural history and possible treatment) of the full range of eye complications secondary to irradiation of periocular tumors may reduce functional and morphological consequences to the eye.

■ 3141

Hypoxia/Reoxygenation and TGF-beta2 Increase Hsp27 Expression in Human Optic Nerve Head Astrocytes

YU AL, KAMPIK A, WELGE-LUSSEN U

Department of Ophthalmology, Ludwig-Maximilians-University, Munich

Purpose Reactive astrocytes in glaucomatous optic nerve changes are characterized by an increased expression of heat shock proteins such as heat shock protein 27 (hsp27). In the pathogenesis of glaucomatous optic nerve damage, ischemia/reperfusion injury and transforming growth factor-beta 2 (TGF-beta2) may play an important role. The goal of the present study was to determine the influence of hypoxia/reoxygenation and TGF-beta2 on the expression of Hsp27 in cultured human astrocytes of the optic nerve head (ONH).

Methods Primary astrocytes were isolated from five human donor eyes. Highly confluent cultured human astrocytes were incubated under hypoxic conditions (1% O₂ for 4 hours) with subsequent reoxygenation (12 to 48 hours). Additionally, cells were treated with 1.0 ng/ml TGF-beta2 for 12 to 48 hours. Expression of Hsp27 was examined by real-time PCR and western-blotting.

Results Hypoxia/reoxygenation increased the expression of Hsp27 at the mRNA (1.3 to 2 fold) and protein level (1.2 to 1.6 fold). Treatment with 1.0 ng/ml TGF-beta2 for 12 to 48 hours markedly enhanced Hsp27 mRNA approximately 2.8 to 5.1 fold. Using Western blot analysis this increase ranged between 2 to 3 times.

Conclusion The process of hypoxia/reoxygenation and TGF-beta2 are capable of inducing the expression of Hsp27 in cultured ONH astrocytes. Therefore, optimization of conditions leading to hypoxia/reoxygenation and blocking of TGF-beta2 action in the ONH of glaucomatous patients may help to lower the incidence of characteristic changes in the optic nerve.

■ 3142

Correct gene dosage of Pax2 is important for normal retinal astrocyte differentiation and normal vascular development in the retina .

SCOTT A (1, 2), BISCOE MP (1), WOLFF J (1), MOSS S (1), GREENWOOD J (1), FRUTTIGER M (1)

(1) Institute of Ophthalmology, London

(2) Moorfields Eye Hospital, London

Purpose To study the Role of Tlx and Pax2 in Astrocyte and Vascular Development in the Retina in the mouse model and to relate this to PAX2 mutations in humans.

Methods Tlx is an orphan nuclear receptor expressed in developing astrocytes and known to be important in regulating the proliferation of precursor cell populations in the retina. It has previously been shown that Tlx is expressed by immature astrocytes that migrate from the optic nerve onto the inner surface of the retina. Immunohistochemistry on Tlx knock-out (KO) mice shows abnormal astrocyte phenotype including premature differentiation, GFAP upregulation, abnormal migration and disordered astrocyte and vascular network interaction. Since it is known that Tlx is an upstream regulator of the Pax2 signaling cascade and Pax2 is normally expressed in retinal astrocytes we studied Pax2 expression in retinal astrocytes.

Results There was no detectable Pax2 in Tlx KO mice. Pax 2 is a transcription factor that plays a major role during embryogenesis. Homozygous deletion of Pax2 is embryonic lethal and we therefore studied heterozygous Pax2 KO mice. We demonstrate that Pax2 heterozygous mice at post natal day 7 have a similar retinal astrocyte phenotype to Tlx KO mice.

Conclusion This suggests that correct gene dosage of Pax2 is important for normal retinal astrocyte differentiation and normal vascular development in the retina. Furthermore we conclude that the retinal astrocyte phenotype observed in Tlx KO mice is, at least in part, caused by an absence of Pax2 expression.

■ 3143

The avascular area in the OIR mouse model: Pathogenesis and kinetics

LANGE CAK, MARTIN G, EHLKEN C, HANSEN LL, AGOSTINI HT

University Eye Hospital, Freiburg

Purpose The oxygen induced retinopathy (OIR) mouse model is frequently used to investigate physiological and pathological angiogenesis. Focus of this study was to analyze the development of the oxygen induced central avascular area as well as the formation of retinal vessels during and after high oxygen exposition.

Methods A total of 130 mice (C57BL/6) were exposed to 75 % oxygen from postnatal day 7 (P7) to P12. At different time points during and after oxygen exposition retinal vessels were visualized by perfusion with fluorescein-dextran and isolectin staining. The size of the developing central avascular area and the total area of retinal vascularization was measured on flatmounted preparations. Also, apoptosis within the retinal tissue was studied by using a TUNEL apoptosis kit.

Results Within two days, high percentage oxygen leads to a maximum size of the central avascular area ($41 \pm 4\%$) via apoptosis of endothelial cells (ECs). At the same time vascularization of the periphery continues, though in a decelerated way compared to a control group. The revascularization of the central area starts two days after the beginning of oxygen exposure via angiogenesis from the vascular periphery and from venous vessels and leads to pathological neovascularisation. On P25, the retinal revascularization is completed.

Conclusion The influence of high percentage oxygen on retinal angiogenesis and ECs depends on their position within the retina. Central vessels undergo apoptosis due to hyperoxia, whereas peripheral vessels seem to be protected and continue to perfuse the periphery of the retina.

■ 3144

Melanocyte Stimulating Hormone Induces Pigmentation of Human Mesenchymal Stem Cells

VOSSMERMERBAUMER U (1), KUEHL S (1, 2), KERN S (2), KLUIETER H (2), JONAS JB (1), BIEBACK K (2)

(1) Department of Ophthalmology, Mannheim Medical Faculty, University of Heidelberg, Mannheim

(2) Institute for Transfusion Medicine and Immunology, German Red Cross Blood Donor Service Baden-Württemberg - Hessen, Mannheim Medical Faculty, University of Heidelberg, Mannheim

Purpose Mesenchymal stem cells (MSC) from adipose tissue have been shown to differentiate into various cell types of the mesodermal lineage. We demonstrated earlier their plasticity for the expression of typical RPE markers. The present study determines basic functional features of RPE in stimulated MSC-derived cells, assessing their response to Melanocyte stimulating hormone (MSH).

Methods Human MSC were isolated from liposuction material, differentiated towards RPE features using RPE conditioned medium (CM) and vasointestinal peptide (VIP) and characterized as described earlier. These cells were subsequently exposed to 10-5 mM α -MSH for 3 days. The response was assessed by light microscopic analysis of pigmented granula in the preparations and quantification of 5 randomly selected, non-contiguous fields of vision.

Results No melanin synthesis of MSC was observed at relevant levels prior to α -MSH stimulation ($2.4 \pm 2.3\%$). α -MSH exposure induced the appearance of pigmented granula in $34.9 \pm 8.7\%$ of undifferentiated MSC. In cells following differentiation culture however pigmentation was increased to $75.7 \pm 2.3\%$ (CM) and $80.3 \pm 0.4\%$ (CM + VIP), respectively.

Conclusion Primary MSC are virtually devoid of pigment yet do show the capacity to respond to α -MSH stimulation. Following induction of differentiation they display a twofold increase in the susceptibility for MSH stimulated pigmentation. It remains subject to further ongoing research to determine whether these in vitro data correspond to in vivo conditions.

■ 3145

Integrin $\alpha 5\beta 1$ ($I\alpha 5\beta 1$) in Choroidal Neovascularization (CNV) associated with Age-Related Macular Degeneration (AMD)

LOEFFLER KU (1), VOGEL A (1), ZAHN G (2), HOLZ FG (1)

(1) *Ophthalmology, University of Bonn, Bonn*

(2) *Jerini AG, Berlin*

Purpose To determine the presence and evaluate the possible role of $I\alpha 5\beta 1$ in neovascular tissue associated with (AMD).

Methods Immunohistochemistry was performed on paraffin sections of 8 CNV membranes removed during subretinal surgery for AMD, using 3 different antibodies (Abs) to $I\alpha 5\beta 1$ as well as Abs to Factor VIII, CD68 and CK 18 on adjacent sections for comparison. As a positive control for $I\alpha 5\beta 1$, tissue from 3 pyogenic granulomas (PG) was used. Furthermore, sections from 6 eyes enucleated for end-stage glaucoma were also examined, in particular with regard to the presence of $I\alpha 5\beta 1$ in normal choroidal vessels and choriocapillaries (CC).

Results In PG, there was intense labeling with anti- $I\alpha 5\beta 1$ of all endothelial cells as well as some macrophages/inflammatory cells. Sections from the enucleated eyes all revealed some anti- $I\alpha 5\beta 1$ immunoreactivity (IR) in normal choroidal vessels while choriocapillaries were mostly negative. In CNV, anti- $I\alpha 5\beta 1$ labeling was seen in most vascular structures and was more prominent than in normal CC but less intense compared to labeling with anti-Factor VIII. Individual cells, most likely of macrophage origin, were also stained but even more prominent labeling was found in retinal pigment epithelial (RPE) cells within these membranes. No significant labeling of RPE cells in glaucomatous eyes was seen.

Conclusion New vessels in CNV exhibit more prominent anti- $I\alpha 5\beta 1$ IR compared to normal CC. The difference appears even more striking with regard to RPE cells from AMD lesions compared to normal RPE. From our findings we therefore conclude that an upregulation of $I\alpha 5\beta 1$ in RPE cells might play an important role in the development of CNV associated with AMD.

■ 3151

Schirmer test and thyroid disease

CHATZIBALIS T (1), SKRIAPA-MANTA A (1), KARAMPATAKIS V (1), RAPTOU E (2), KALATHAS T (2), SIABANOPOLIOU M (2), KARAMITSOS A (1)
 (1) Laboratory of Experimental Ophthalmology, Aristotle University, Thessaloniki
 (2) Nuclear Medicine Department, "Theagenvion" Cancer Institute, Thessaloniki

Purpose To investigate the tear secretion in thyroid patients.

Methods 47 consecutive thyroid patients who underwent thyroidectomy due to cancer or having hyperthyroidism (aged from 40 to 72 years - mean 55.4, 40 females and 7 males) were recruited for this study. These patients were frequently examined (RIA methods) for their thyroid status (26 euthyroids, 12 hypothyroids and 9 hyperthyroids). Some of them were under treatment (substitution or suppression) and some of them free of treatment. 36 persons of age-matched non-thyroid patients and without any pathology affecting tear tests were examined as control subjects. Schirmer test (without anesthetic) was performed the day that the patients underwent routine exams for thyroid hormones. Only the right eyes of the patients were included for analysis. Abnormal (impaired secretion) for classification of Schirmer test results were considered values of ≤5 mm in 5 min.

Results 13 (50%) of the euthyroids had values ≤5mm. 5 (42%) of the hypothyroids had values ≤5mm. 5 (56%) of the hyperthyroids had values of ≤5mm. As regards the control group 4 individuals (11%) had values ≤5mm.

Conclusion Thyroid disease patients who underwent or not thyroidectomy, being or not being under treatment, are prone to eye dryness although they may be euthyroids.

■ 3152

Ocular Surface Bacterial Flora Identification- A Conundrum

GRAHAM JE (1), MOORE JE (2), XU J (3), DOOLEY JS (1), HAYES V (1), DENG W (1), MOORE CBT (1), MOORE JE (4)
 (1) CMB UU, Coleraine
 (2) RVH, Belfast, Belfast
 (3) BCH, Belfast
 (4) Belfast City Hospital, Belfast

Purpose The ocular surface is known to support a diverse range of bacteria existing as normal commensal organisms on the lid margins and on the mucosa of normal healthy individuals. The concentration and type of bacteria present at any given time can change with various ocular surface conditions. The aim of this study was to investigate the range of bacterial types present on the ocular surface of normal quiet eyes vs those with mild-moderate dry eye.

Methods Conjunctival samples obtained from bulbar conjunctiva of 91 subjects underwent bacterial identification using culture techniques or 16S rDNA PCR, cloning and sequence analysis.

Results A consistently higher incidence of bacteria was detected on the conjunctiva and lid margins of dry eye subjects. The bacterial population of the ocular surface of many subjects was restricted to a single genus. 86% of all bacterial isolates were identified as *S. epidermidis* while 8% were determined to be *S. lentus* and 3% *S. xylosus* and *Bacillus* sp. Bacterial species including *Rhodococcus erythropolis*, *Bacillus* sp., *Corynebacterium*, *Propionibacterium*, *Klebsiella* sp. and uncultured bacteria were detected in a number of samples through sequencing while remaining undetectable in routine culture. *Rhodococcus erythropolis* (99% identity) was identified in 4 subjects of which 3 were normal subjects. Likewise *Klebsiella oxytoca* was detected in 4 subjects, 2 of which were DE and using artificial tears.

Conclusion It remains uncertain the role, if any, these potentially pathogenic bacteria play on the ocular surface of the normal or dry eye and as such these findings present a dilemma regarding management.

■ 3153

Efficacy of an Antibody Based Assay for the Detection of Herpes Simplex Keratitis

ANWAR ST (1), MOORE JE (2), YEUNG I (1), GARDINER T (2), SHARMA A (3), FRAZER DG (4), JACKSON AJ (2), SHAH S (5), MOORE CBT (1)
 (1) University of Ulster, Coleraine
 (2) Department of Ophthalmology, RVH, Belfast
 (3) Department of Ophthalmology, Bedford Hospitals NHS Trust, Bedford
 (4) Department of Ophthalmology, RVH, Belfast
 (5) BMEC, Birmingham

Purpose Herpes Simplex Keratitis (HSK) a sight threatening ocular infection requiring specific and prompt diagnosis, enabling appropriate patient management to prevent an inappropriate lifelong label of HSK. Currently diagnosis of HSK relies on clinical identification of dendritic ulcers. A specific and rapid diagnostic test would improve patient care enabling appropriate treatment. This study investigated the efficacy of an antibody based assay for the detection of Herpes antigen in ex-vivo human models of HSK.

Methods Corneal rims or primary human corneal epithelial cells were infected with HSV-1 or HSV-2 and monitored for cytopathic effect, while uninjected corneal tissue or cells served as negative controls. Direct immunohistochemical analysis using the anti-gp C antibody (Biosynergy Ltd) or negative control antibody (anti-DM) was used to detect the presence of viral antigen with the aid of confocal scanning laser microscopy.

Results The anti-gp C fluorescent mAB demonstrated specific cell surface and cytoplasmic staining in HSV-1 infected corneal rims and cells. No fluorescence was noted on HSV-2 infected or uninjected control corneal rims with either the Herpes antibody or with the control anti-DM antibody in any of the samples tested as expected (n=6).

Conclusion This in vitro study on human corneal tissue and cells demonstrated the specificity and sensitivity of this monoclonal antibody to detect viral antigen in models of HSK infected corneas. Further studies are ongoing through the use of in vivo confocal microscopy assessing the use of this antibody on the ocular surface of patients presenting with HSK. This may prove to be a useful clinical tool for rapid and accurate in vivo diagnosis of HSK.

■ 3154

Nodular Degeneration

WOOD TO, WILLIAMS EE, HAMILTON DL
Associated Ophthalmic Specialists, Inc., Germantown

Purpose To describe the clinical symptoms, surgical procedure, and results of patients with symptomatic nodular degeneration.

Methods Thirty eyes in 25 patients with symptomatic nodular degeneration were included in this study. Twenty-one were women. Twenty-two of the eyes presented with discomfort and decreased vision, five with decreased vision, and three with only irritation. Twenty-nine underwent removal of the nodules using topical anesthesia and toothed forceps, at the slit lamp, without laser surgery; one patient with extensive corneal involvement had surgery in the operating room.

Results Twenty-seven of the 30 eyes have follow-up at the present time (average follow-up, 10.9 months). Twenty-six of the 27 had improvement in discomfort, vision, or both. One patient has had no improvement. None of the patients have required re-treatment.

Conclusion Simple dissection of nodular degeneration from the corneal surface without the addition of eximer laser treatment provides excellent results. The operation can usually be completed in 10 minutes or less as a slit lamp procedure.

■ 3155 / 335

Improvement of dry eye symptoms with polyunsaturated fatty acids

GARCHER CREUZOT C (1), PASSEMARD M (1), LAFONTAINE PO (1), VIALS S (2), JOFFRE C (2), ELENA PP (3), POULIQUEN P (4), BALIDOUIN C (5), BRON AM (1)

(1) Department of Ophthalmology, Dijon

(2) Eye and Nutrition Unit - INRA, Dijon

(3) Cliropta, Nice

(4) Thea Laboratories, Clermont-Ferrand

(5) Department of Ophthalmology - CHNO XV-XX, Paris

Purpose Polyunsaturated fatty acids (PUFA) are involved in inflammatory pathways via prostaglandins. Conjunctival inflammation is a hallmark of all dry eye syndromes. We investigated the role of dietary n-6 and n-3 fatty acids in patients suffering from ocular dryness with peculiar attention to inflammatory markers.

Methods 71 patients presenting with mild to moderate dry eye syndromes were randomly assigned to Nutrilarm® or placebo pills, twice a day for 6 months. Schirmer test, BUT, fluorescein and lissamin green stainings were performed at inclusion and after 1, 3 and 6 months. Impression cytology to evaluate MHCII antigens was harvested at D0, Month 3 and 6. Tear PGE1 levels were measured at D0, Month 1, 3 and 6. Furthermore, a questionnaire related to the dry eye symptoms and global discomfort was provided at every visit.

Results Schirmer test, BUT, fluorescein and lissamin green stainings were improved with treatment when compared to placebo but the difference remains non statistically significant. We found the same trend with MHCII and prostaglandin tear secretion as well as discomfort feeling ($p=0.091$). Efficacy evaluated by the patients and the investigator was nearly significant ($p=0.052$ and $p=0.054$ respectively). For some signs, such as reflex tearing and conjunctival hyperaemia, the improvement was nearly significant ($p=0.047$ and 0.045 , respectively). The same results were found with skin quality and emotional condition which were improved (61% with treatment vs 36% with placebo).

Conclusion This double-masked, pilot study shows that PUFA seem an interesting tool to alleviate the symptoms related to dry eye syndrome. These results should be confirmed using a larger study population.

■ 3157 / 337

Addition of ganciclovir to the cornea preservation medium : kinetics and toxicity

ROBERT PY (1), SAUVAGE FL (2), THURET G (3), RANGER-ROGEZ S (4), ADENIS JP (1), ROUSSANE MC (5), GAIN P (3), MARQUET P (2)

(1) Ophthalmology, Limoges

(2) Pharmacology, Limoges

(3) Ophthalmology, Saint-Etienne

(4) Virology, Limoges

(5) Tissue Bank, Limoges

Purpose HSV-related primary endothelial necrosis has highlighted the possible transmission of HSV from donor to recipient in human keratoplasty. We investigate the pretreatment of donor cornea during preservation by addition of ganciclovir in the preservation medium.

Methods 2mg/L, 10 mg/L or 20mg/L ganciclovir is added in the preservation medium of human corneas excluded from the graft process for serological reasons. Ganciclovir concentration is tested in preservation medium and in corneal tissue every 8 days for 3 weeks. Corneal toxicity is assessed by trypan blue staining.

Results Ganciclovir concentration undergo linear decrease during preservation, but remains above CI 50 fo HSV after 3 weeks in all cases. Corneal transparency remains normal during the whole process. Endothelial toxicity is discussed.

Conclusion Ganciclovir supplementation of preservation medium will increase viral security of corneal grafts. This is the first step in the way to certification and in vivo experiments.

■ 3156 / 336

Ultrastructural Conjunctival Surface Changes in Patients with Sjögren's Syndrome: Long term Results

KOLIFAKIS DI (1), KARABATSAS CH (2), SAKKAS LI (3), ALVANOU A (4), MANTHOS AK (4), CHATZOULIS DZ (2)

(1) University of Thessaly Medical School, Larissa

(2) Dept. of Ophthalmology, University Hospital of Larissa, Larissa

(3) Dept. of Rheumatology, University Hospital of Larissa, Larissa

(4) Laboratory of Histology, Embryology, Anthropology, Aristotle University of Thessaloniki, Thessaloniki

Purpose To demonstrate the ultrastructural appearance of the conjunctival surface epithelium in patients with Sjögren's syndrome (SS) compared with normal subjects.

Methods Conjunctival tissue specimens from 13 normal subjects and 32 patients with SS were obtained by bulbar conjunctival biopsy, and examined by transmission electron microscopy.

Results The average number of microvilli per 8.3 μm epithelial length was significantly lower in the SS group than that in controls (16.28 ± 5.72 vs. 28.92 ± 3.09 , $P < 0.01$). The microvillus height ($0.487 \pm 0.163 \mu\text{m}$) and height/width ratio (1.587 ± 0.49) in the conjunctival epithelium in the SS group were significantly lower than those (height: $0.939 \pm 0.093 \mu\text{m}$, $P < 0.01$; and height/width ratio: 3.740 ± 0.511 , $P < 0.01$) in normal individuals. The microvilli in the SS group were wider than those in the control group ($P = 0.046$). Furthermore, the average number of secretory vesicles (per 8.3 μm epithelial length) in the apical conjunctival epithelial cell was significantly reduced in the SS group (15.77 vesicles ± 5.77), compared to controls (33.5 vesicles ± 2.07 , $P = 0.01$). In addition, while the OSG was always present in controls, this was not detectable in all but one SS conjunctival specimens.

Conclusion The ultrastructural morphology of the apical conjunctival epithelium is altered in patients with SS. Our findings suggest that an intact OSG may play a key role in the maintenance of a healthy ocular surface possibly by preventing abrasive influences on the apical epithelial cells.

■ 3158 / 338

Bevacizumab (Avastin) inhibits inflammatory hem- and lymphangiogenesis in the cornea

BOCK FB, ONDERKA J, DIETRICH T, BACHMANN B, KRUSE FE, CURSIEFEN C

Department of Ophthalmology, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen

Purpose Intention of this work was to analyze whether Bevacizumab can inhibit inflammatory angiogenesis and in addition also lymphangiogenesis in the cornea. Bevacizumab (Avastin®) is a recombinant, humanized, monoclonal antibody against VEGF-A, FDA-approved for the treatment of colon carcinomas in 2004.

Methods The mouse model of suture induced corneal neovascularization was used to assess the antihem- and antilymphangiogenic effect of Bevacizumab by systemic and topical application. Corneal flat mounts were stained with LYVE-1 as a specific lymphatic vascular endothelial marker and CD31 as panendothelial marker and blood- and lymph-vascularised areas were analyzed morphometrically. The inhibitory effect of Bevacizumab on lymphatic endothelial cells (LEC) was analyzed with a colorimetric (BrdU) proliferation ELISA. The binding capacity of Bevacizumab to murine VEGF-A was analyzed using Western Blot.

Results The systemic/topical application of Bevacizumab significantly inhibited the outgrowth of blood ($p<0.006$ / $p<0.0001$) and lymphatic ($p<0.002$ / $p<0.0001$) vessels. Inhibition of the proliferation of LECs was also significant ($p<0.0001$). Western Blot analysis showed, that Bevacizumab binds to both human as well as murine VEGF-A.

Conclusion Bevacizumab inhibits both inflammation-induced angiogenesis as well as lymphangiogenesis in the cornea by topical as well as systemic application. This suggests an important role of VEGF-A in corneal lymphangiogenesis. Bevacizumab may be useful in preventing immune rejections after penetrating keratoplasty or tumor metastasis via lymphatic vessels.

■ 3159 / 339

Use of Platelet-Rich Plasma In The Treatment of Ocular Surface Syndrome Following Laser In Situ Keratomileusis (LASIK)

PASTOR S (1), RUIZ-COLECHA J (2), RODRIGUEZ A (1), ARTOLA A (2), ALIO JL (2)

(1) Research, Development and Innovation. Instituto Oftalmológico, Alicante
(2) Ophthalmology. Instituto Oftalmológico, Alicante

Purpose To treat patients suffering from ocular surface syndrome following Laser in situ Keratomileusis (LASIK) with Platelet-Rich Plasma.

Methods Autologous PRP was used in a consecutive series of 20 cases with significant dry eye symptoms following corneal refractive surgery with punctate keratitis at the slit lamp examination. Selected cases were followed by impression cytology.

Results Autologous PRP was useful and effective in decreasing subjective symptoms and improving visual performance in 50% of the cases, 25% showed moderate improvement, improving also the slit lamp appearance, and in 20% of the cases the treatment was ineffective. The study of selected cases by impression cytology showed that significant changes appeared in the cytology pattern following 1 month, and especially 2 months of treatment.

Conclusion Autologous PRP might be a useful tool in the management of cases of ocular surface syndrome following LASIK.

■ 315a / 334

Correlation between impression cytology and Labial salivary gland biopsy in Sjögren's syndrome

LANGMAN ME (1, 2), GICQUEL JJ (2), ROBLOT P (3), GOMBERT JM (4), DIGHIERO PL (2)

(1) St Louis Hospital Ophthalmology Department, Saint Jean d'Angély
(2) Jean Bernard University Hospital Ophthalmology Department, Poitiers
(3) Infectious Diseases / Jean Bernard University Hospital, Poitiers
(4) Immunology / Jean Bernard University Hospital, Poitiers

Purpose To evaluate a possible correlation between impression cytology (IC) and histopathological findings obtained from salivary gland biopsy in patients with Sjögren syndrome.

Methods Thirty five consecutive patients who were referred on the same day of the salivary gland biopsy, to our department for severe dry eye were enrolled and had complete ophthalmological examination including refraction, tear function testing (break up time, jones test), slit lamp photographs. Two IC specimens per eye were collected for histocytological analysis (Nelson classification) and a study in flow cytometry of the expression of HLA-DR.

Results Thirty patients out of thirty five were diagnosed with Sjögren syndrome. There is a significant correlation between the severity of the histopathological findings from salivary gland biopsies, functional study of the tear film, results obtained from IC specimens with Nelson classification and the overexpression of HLA-DR measured in flow cytometry.

Conclusion A complete ophthalmological examination associated with the study of the conjunctiva with IC may render obsolete the use of gland biopsy in Sjögren syndrome.

■ 3161

The European EVI-Genoret database

POCHO
-, Strasbourg

ABSTRACT NOT PROVIDED

■ 3162

Phenotyping Retinal Degenerations, an European Effort

ZRENNER E (1), WILKE R (1), SCHOLL HP (2)
(1) University Eye Hospital, Tuebingen
(2) Dept. of Ophthalmology, Bonn

Purpose Rapid progress in understanding disease mechanisms in monogenic hereditary and age-related retinal diseases on a molecular and cellular level and the increasing availability of successful preventive and therapeutic strategies in animal models have led to major breakthroughs in the developing treatments for some of these diseases. The application to patients can already be foreseen in the light of clinical studies currently underway or planned for the near future. European scientists and clinicians belong to the vanguard of these developments. With the formation of the European Vision Institute (EVI) a multinational activity was initiated in 2003 to improve transnational cooperation. The EVI-GENORET (www.evi-genoret.org) project ("Functional genomics of the retina") is the largest integrated scientific project on eye diseases ever established in Europe. It involves a consortium of 50 laboratories from 24 different academic and industrial entities located in 12 European countries. One of the objectives of EVI-GENORET is to create the first integrated European clinical database of retinal degeneration. Within this framework, an internet-based patient assessment program has already been created that is based on standard operating procedures and a harmonized case report form, both of which will be presented. These instruments also serve to improve the quality of multicenter studies in ophthalmology, an activity fostered by EVI. CTSE (http://www.europeanvisioninstitute.org/CT_SE/), a cooperation comprising 12 certified ophthalmology departments. Besides collecting and disseminating clinical and biological data this project will focus scattered patient care activities in a strong, interactive European network of high quality and also bridge the gap between basic science and clinical research in the field of diseases affecting photoreceptors and pigment epithelial cells of the retina.

■ 3163

Bringing large scale genotyping into the agenda

BHATTACHARYA SS
Institut of Ophthalmology UCL, Molecular Genetics, London

ABSTRACT NOT PROVIDED

■ 3164

Stem cell differentiation : the importance of functional genomics

MARIGO V (1, 2), FIOCCO R (3), GUILLONEAU X (4), VETRINIF (1),
GOUREAU O (5)
(1) Telethon Institute of Genetics and Medicine, Naples
(2) Department of Biomedical Sciences, University of Modena and Reggio Emilia, Modena
(3) SCRI, Neural Stem Cell unit, Milan
(4) INSERM U592, Paris
(5) Laboratoire de Physiopathologie Cellulaire et Moléculaire de la Rétine, INSERM U592, Paris

Purpose Compare the molecular characteristics of retinal stem cells with retinal progenitors and neural stem cells. Define the identity of the cell types in growing and differentiating cell cultures through gene expression spectrum. Assess the different differentiation potential.

Methods In vitro culture of retinal stem cells derived from the adult ciliary margin and neural stem cells derived from the subventricular zone. Quantitative PCR analysis of retinal neuronal and progenitor specific markers in retinal stem cells, neural stem cells and retinal progenitors at different embryonic and postnatal ages. Immunofluorescence assessment of retinal stem cell differentiation into different retinal cell types.

Results Quantitative PCR was performed on RNA extracted from: stem cells grown as neurospheres, progenitors derived from neurospheres and retinal tissue at different developmental stages. Several retinal markers were analyzed and the relative amount was measured. A subgroup of genes showed a specific expression in stem cells derived from the eye compared to stem cells derived from the brain. Treatment of retinal stem cells with different growth factors changes the molecular characteristics and differentiation potentials of these cells.

Conclusion We have analyzed a panel of retinal markers and found that a group of retinal progenitor specific genes show a differential expression in stem cells derived from the adult eye ciliary compared to neural stem cells derived from the brain. Our studies identified a group of six genes as genetic labels of retinal stem cells. This demonstrates that the early acquisition of molecular characteristics and probably differentiation potentials of neurospheres derived from different neuronal tissues.

■ 3165**Molecular interactions of rhodopsin - from the proteome inventory of the outer segment towards analysis of functional protein networks**

LIEFFING M (1, 2), SWIATEK-DE LANGE M (1), CHATR-ARYAMONTRI A (3), BEER M (1), CESAREN G (3)

(1) *GSF National Research Centre for Environment and Health, Institute of Human Genetics, Munich-Neuherberg*

(2) *Technical University, Munich*

(3) *University of Rome Tor Vergata; Department of Biology, Rome*

Purpose Mutations in the light receptive GPCR rhodopsin, and in proteins linked to its downstream signalling account for numerous blinding diseases. The mechanistic interpretation of the majority of rhodopsin mutations is not straightforward, suggesting that, at least some of them may affect its function indirectly by altering the correct wiring of a large and still poorly understood protein network. With the aim of establishing a protein interaction map centred on rhodopsin, we systematically investigated protein interactions from isolated photoreceptor outer segment discs. We combined biochemical native separation methods with mass spectrometric identification of the isolated (super-)complexes and present an interaction map of rhodopsin based on the analysis of protein complexes recovered in their native form. Out of all interactions, 101 from our dataset are putatively novel. The authenticity of isolated protein interactions was independently cross-validated by either data-mining or experimental proof via a second alternative method. By using the genome wide protein interaction data deposited in the MINT database we have extended the protein network obtained by our approach thereby gaining information on the topology of the complexes. This analysis has also offered a view of the peripheral links between the main rhodopsin pathway and the rest of the cell functional pathways. The dataset suggests that in addition to its role as a light transducing GPCR, rhodopsin plays a role in regulating photoreceptor structure and polarity and is likely to orchestrate a biological network with diverse functional diversifications.

■ 3166**Gene therapy: from bench to benchside**

ALIRR

UCL Institute of Ophthalmology, London

Purpose The eye has unique advantages as a target organ for gene therapy of both inherited and acquired disorders. It is readily accessible to phenotypic examination and investigation of therapeutic effects in-vivo by fundus imaging and electrophysiological techniques. Considerable progress has been made in the development of gene replacement therapies for retinal degenerations resulting from gene defects in photoreceptor cells (peripherin, RPGRIP, retinoschisin) and in retinal pigment epithelial cells (MerTK, RPE65, OA1) using recombinant AAV and lentivirus-based vectors. Gene therapy also offers a potentially powerful approach to the treatment of complex acquired retinal disorders such as those involving angiogenesis, inflammation and degeneration, by the targeted sustained intraocular delivery of therapeutic proteins. One of the most amenable conditions for treatment by gene therapy is a form of childhood onset retinal dystrophy caused by mutations in the gene encoding RPE65 which is required for the conversion of vitamin A to 11-cis retinal by the RPE and thus for the regeneration of the rod visual pigment. The demonstration of long-term functional improvement following gene-replacement of RPE65 in pre-clinical models has supported proposals for clinical trials of AAV-mediated gene therapy for patients with RPE65 deficiency. Proposals for trials have received ethical approval in the USA and UK, and are likely to start later this year.

■ 3167**Towards European Clinical Trials**

CLINHA-VAZ J (1, 2, 3)

(1) *AIBILI, Coimbra*

(2) *Ophthalmology Department of the University Hospital, Coimbra*

(3) *Centre of Ophthalmology and Visual Sciences, Coimbra*

Purpose In the area of Ophthalmology and Visual Sciences, a Network of European Ophthalmological Clinical Research Sites, dedicated to perform clinical research in Ophthalmology with the highest standards of quality, following the European and International Directives for clinical trial research has been created under the umbrella of the European Vision Institute EEIG. This Network is called: European Vision Institute. Clinical Trials. Sites of Excellence (EVI.CT.SE) with the central office located in Coimbra, Portugal.

The EVI.CT.SE is a Special Committee of the European Vision Institute, European Economic Interest Grouping (EVIEEIG) legally constituted in 2003 under European law as a not-for-profit, science-driven organisation in Brussels, to facilitate and encourage cross-border co-operation in Vision Research.

The aims and objectives of EVI.CT.SE are:

- Performance of state-of-the-art multicentric clinical trials in Ophthalmology in Europe
- High level of quality and excellence in the work performed by the members – Uniformized SOPs
- Uniform levels of performance and maintenance of qualified personnel – Training and certification
- Regular recruitment of patients

All Clinical Trial Sites are certified by independent Auditors according to EVI.CT.SE SOPs.

This Network of European Clinical Trial Centres in Ophthalmology is expected to become an important partner in the process of drug development in Europe.

■ 3211

Bioinformatic Approaches to Retinal Gene Expression

ZACK D, YUXI QIAN J

Departments of Ophthalmology, Molecular Biology and Genetics, and Neuroscience, Johns Hopkins University School of Medicine, Baltimore

Purpose Multiple transcription factors (TFs) work in concert to achieve tissue specificity. In an attempt to understand TF interactions, with particular emphasis on those that are relevant to retina-specific gene expression, we are using a bioinformatics approach to identify biologically relevant interactions.

Methods We first identified tissue-specific genes for 30 tissues. We then searched the evolutionarily conserved regions in the human genome using 306 DNA binding matrices available from TRANSFAC and the literature. The interaction between each pair of TFs was evaluated according to the distances between their respective binding sites on the promoters and the specificity of the pair to the tissue-specific gene groups.

Results TF interactions in 30 tissues were derived. Known protein-protein interactions are highly enriched among the predicted interactions (>60 times that of random expectation). For genes preferentially expressed in the eye, approximately 100 interacting TF pairs were predicted, including CRX:CRX, CRX:NRL, CRX:CHX10, and NR2E3:MEF2. Since CHX10 has been suggested to act as a repressor of CRX-mediated activation of rhodopsin in non-photoreceptor retinal cells, the finding of a CRX/CHX10 interaction is interesting because it suggests that the algorithm identifies negatively interacting as well as positively interacting TF pairs. We also examined the gene expression of the target genes of the interacting TF pairs, which may reflect the activity of the TF pairs. We found that the eye specific TF interactions are, in general, only active in the eye, but not in the other tissues.

Conclusion A bioinformatics approach can be used successfully to predict interactions between TFs. Such information can complement experimental studies of gene regulation, and we are in the process of experimentally testing some of the bioinformatic predictions related to retinal gene expression.

■ 3212

Two novel mouse models with retinal degeneration

DALKE C (1), PIUK O (1), HRABÉ DE ANGELIS M (2), GRAW J (1)

(1) Institute of Developmental Genetics, Neuherberg

(2) Institute of Experimental Genetics, Neuherberg

Purpose To establish new disease models with retinal degenerations an ENU mutagenesis screen was performed, beside screening mice for spontaneous mutations.

Methods Indirect funduscopy and ERG are used to recognize alterations in the retina. To generate mutants, C57BL/6 males are treated with ENU. The offspring are examined for retinal impairment and variants are tested for heredity.

Results In our colony of JF1 mice, some animals (rei1) with less than half of the normal ERG amplitudes of a- and b-waves were identified. In the fundus big white areas were observed, often just in one eye. Retinal degeneration with an individually varying phenotype was confirmed by histological analysis. The retinal layers showed disturbed lamination, mainly of the ONL. In some animals also a doubling of photoreceptor cell layers or the formation of rosette like structures was seen, as well as areas with a very thin retina, mainly affecting the INL to a total degeneration of all layers. In the offspring the same fundus phenotype is expressed, but not in the expected frequency, indicating a low penetrance and/or the participation of several genes. In a recessive ENU mutagenesis screen, about 1000 mice were examined and 49 mice with a fundus phenotype were picked up as putative mutants. Up to now one mutant line (fun1) was confirmed. Four of six mice of the confirmation cross showed the expected alterations. The phenotype of this line is characterized by wide red streaks with a diffuse border in the retinal fundus. Further details of the ongoing morphological and genetical analysis will be discussed.

Conclusion Two novel mouse lines with a retinal impairment were established serving as new disease models. This work is supported by the NGFN.

■ 3213

Screening for new mouse mutants affected in eye development using the OLCI technique

PIUK O, DALKE C, GRAW J

GSF-National Research Center for Environment and Health, Institute of Developmental Genetics, Neuherberg

Purpose Aim of the study is to select new mouse mutants characterized by altered sizes of ocular tissues.

Methods Offspring from ENU-treated C57BL/6J mice were analyzed with optical low coherence interferometry (OLCI) at the age of 11 weeks. Putative eye-size variants were selected and genetically confirmed.

Results OLCI has been shown to be an effective tool for the measurement of the size of the mouse eye (Schmucker and Schaeffel, Vis. Res. 2004; 44:2445-56). In order to enable the recognition of eye-size mutants, we first determined the biological variation of axial length, anterior chamber depth and lens size in wild-type mice (Puk et al., Mamm. Genome; in press). Up to date, 430 offspring from ENU-treated C57BL/6J mice have been analyzed. 36 of them were selected as putative eye size variants (Aca1-Aca36). Confirmation crosses established the variants Aca8, Aca12, Aca15 and Aca16 as stable mutant lines. Aca8 is characterized by a non-mendelian inheritance of abnormalities concerning axis and lens, which individually range from 4.9% smaller to 4.7% larger eyes than in wild-type animals. In Aca12, axes and/ or lenses are significantly smaller (2.1% - 7.6%) than in controls, while in Aca15 mice only reduced lens sizes (3.0% - 8.9%) occur. Moreover, Aca16 offspring exhibit enlarged axial lengths (2.8% - 4.2%); these mice might be considered as glaucoma models. Mapping studies are in progress.

Conclusion 36 putative eye size variants were selected in an ongoing ENU-screening program. Four of them were identified as stable mutants and will be analysed in mapping studies.

■ 3214

Phenotypic assessment of a novel murine ENU-induced mutant line expressing a nonsense mutation in the opa1 gene

DAVIES VJ (1), PIECHOTA MJ (1), HOLLINS AJ (1), VOTRUBA M (1, 2)

(1) School of Optometry & Vision Sciences, Cardiff University, Cardiff

(2) Cardiff Eye Unit, University Hospital Wales, Cardiff

Purpose Autosomal dominant optic atrophy caused by over 100 mutations, predominantly in the opa1 gene, is characterised by progressive loss of visual acuity and atrophy of the optic nerve through loss of retinal ganglion cells (RGCs). To examine the mechanism behind RGC loss we generated and visually phenotyped a mouse line expressing a protein truncating mutation in the opa1 gene.

Methods A hybrid heterozygous mutant mouse line expressing a nonsense mutation in the opa1 gene was generated through ENU mutagenesis. To assess for a functional visual phenotype, 12 F1 opa1^{+/-} mice with appropriate controls were tested at 6-12 months of age on the SHIRPA neurological primary screen, 3 different sized gratings (2, 4 & 8 degree) in the optokinetic drum (OD), and on the circadian running wheel (CRW) during their dark cycle.

Results The heterozygous opa1^{+/-} mice showed no signs of gross systemic or neurological abnormalities on the primary screen at any age tested. At 12 months of age the opa1^{+/-} mice tracked all gratings in the OD, but less often than WT controls ($F(1, 26) = 14.204, p < 0.05$), and continued their CRW activity on presentation of a light source during their dark cycle, whereas the control mice stopped ($U(12, 12) = 37, p < 0.05$).

Conclusion Results from the optokinetic response indicate that opa1^{+/-} mice have a mild functional visual deficit. The abnormal running wheel response suggests that there is an abnormality in circadian rhythmicity which may reflect loss of specific RGCs. The presence of subtle functional visual loss, without other neurological abnormality, highlights the need to enhance the phenotype through ageing and stress, and is in line with the heterogeneous nature of this condition in man.

■ 3215

Polymorphism in PITX2 is associated with Open Angle Glaucoma

VAIDEANU D (1, 2), SOWDEN J (3), FRASER S (1)

(1) Eye Infirmary, Sunderland

(2) Institute of Human Genetics, Centre for Life, Newcastle upon Tyne

(3) Institute of Child Health, London

Purpose Mutations in the PITX2 homeobox transcription factor gene disrupt normal development of the anterior segment and cause overt structural abnormalities. It is also possible that, as yet undetected mutations/polymorphisms in PITX2 may produce undetected abnormalities in anterior segment structure and function that could predispose to glaucoma. The purpose of this study was to screen a cohort of patients with open angle glaucoma (OAG) for mutations/polymorphisms in the PITX2 gene to establish the mutation spectrum.

Methods 105 POAG patients and 60 age and ethnically matched controls were enrolled in the study. Patients and controls were phenotyped. Polymorphic sites reported in the PITX2 gene were identified from the NCBI (<http://www.ncbi.nlm.nih.gov/SNP/>) and Ensembl (http://www.ensembl.org/Homo_sapiens/geneview?db) database. The MassARRAYTM Discovery-RT(SNP Discovery) was used to investigate the frequency of polymorphic sites. The SHEsis software package was used for statistical analysis.

Results 26 polymorphic sites in PITX2 were identified from the databases, including five in the coding sequence. Sixteen non coding SNPs were confirmed within our study group and SNP frequencies were examined. One SNP was found at a significantly increased frequency within the OAG group compared to the control group (rs 2739202, p=0.01).

Conclusion One SNP (rs 2739202), located at the 3'UTR end was significantly more prevalent among OAG group compared with controls (p=0.01). To our knowledge, this is the first report of an association of PITX2 polymorphism with OAG. The implications of this finding will be discussed.

■ 3216 / 376

Delayed development of Retinal Vessel Network in Alpha 2B Adrenergic Receptor Knock-out Mice

HUA J (1), MUTHIG V (2), GROSS N (1), LANGE CAK (1), MARTIN G (1), HANSEN LL (1), HEIN L (2), AGOSTINI HT (1)

(1) University Eye Hospital, Freiburg

(2) Institute of Experimental and Clinical Pharmacology and Toxicology, University of Freiburg, Freiburg

Purpose Alpha 2 adrenergic receptors (Adra2) mediate diverse biological effects of the endogenous catecholamines epinephrine and norepinephrine. Subtype Adra2b was found to play an essential role for placental vascular development, whereas the other two subtypes Adra2a and c are presynaptic feedback regulators of sympathetic nerves. To determine its role in vasculogenesis and angiogenesis, we studied the development of retinal vasculature in Adra2b knock-out (Adra2b^{-/-}) and wild type C57Bl/6 mice.

Methods Adra2b^{-/-} and C57Bl/6 mice underwent intraventricular perfusion with FITC-dextran solution. After enucleation, retinal flat mounts were prepared at different time points: first (p1), fourth (p4), seventh (p7) and tenth (p10) postnatal day (n=4 per time point and genotype). The ratio of vascularised area to total retina was taken as an index for completed vascularisation.

Results There is significant delay of vascularisation in Adra2b^{-/-} mice on p4 and p7 compared to the control group. Both in Adra2b^{-/-} mice and C57Bl/6 mice about 5% of the central retina is vascularised on p1. The vessel network reaches the edge of the peripheral retina on p10 in both genotypes.

Conclusion The development of the murine retinal vasculature is influenced by Adra2b. However, the cellular mechanisms involved remain unknown. Recent studies indicated that activation of the mitogen-activated protein kinase (MAP kinase) pathway by Adra2 is required for placenta and yolk-sac vascular development. Future studies will have to address whether the findings derived from knockout mice are relevant for human physiology and retinal disease.

■ 3217 / 377

Temporal expression profile of murine cornea: a possible role for Vsx1 in corneal development?

SHEPPARD JPB (1), MEEK KM (1), VOTRUBA M (1, 2)

(1) Optometry and Vision Sciences, Cardiff

(2) Cardiff Eye Unit, University Hospital Wales, Cardiff

Purpose To examine the expression of a number of corneal genes, including the paired-like homeobox gene Vsx1 in postnatal mice during eye lid opening in order to study the role of these genes in corneal development.

Methods Whole murine eyes, brain, liver, lung and heart from 4 mice were extracted and placed in RNAlater at P5, 12 and 17 of development. Eight corneas at each time point were dissected out and extracted RNA was quantified and transcribed to cDNA for quantitative PCR. cDNA was amplified with SYBR Green for real-time quantitative PCR using RNA specific primers for the following genes: Vsx1, Aqp1, Aqp5, and Col8A2. Rpl19 was used as a housekeeping gene for normalization.

Results Vsx1 shows a ~2 fold increase in expression from P12 to P17 in the cornea, while expression was absent at P5. Vsx1 expression was also found in the brain and liver at P12 and in the brain, liver and lung at P17. Expression of Aqp5 has a ~250 fold higher expression and Aqp1 has a ~3.8 fold increase in expression from P12 to P17. Col8A2 shows highest expression at P5 with a subsequent 2.4 fold decrease at P12 and 2.83 fold decrease at P17.

Conclusion The period of eyelid opening in murine development sees many changes especially in corneal thickness. Mutations in Vsx1 have been implicated in keratoconus, a corneal thinning ectasia and the gene Col8A2 has been linked with PPCD. Our data indicate that expression of the aquaporin genes examined increases from P12 to P17. Of note is that Vsx1 expression is also increased from P12 to P17 while expression of Col8A2 decreases at the same time points. As both these genes have been implicated in PPCD there is the possibility that these two genes interact, which could merit future study.

■ 3221

Role of Botulinum toxin A in non-paralytic strabismus

OZKAN SB

Adnan Menderes University Medical School Department of Ophthalmology, Aydin

Purpose Botulinum toxin A (BTA) is a widely used agent in management of certain strabismus disorders. The contribution of BTA in non-paralytic strabismus management will be highlighted in this lecture.

Methods Botulinum toxin A is used both for diagnostic or therapeutic purposes in concomitant strabismus.

Results As a diagnostic agent BTA is used in patients with postoperative diplopia risk and in cases with acquired loss of fusion. For therapeutic purpose BTA may be used alone or as an adjunct to surgical treatment. In general it is an appropriate treatment in patients with small angle deviations and especially in those with previous surgeries and in all cases where surgery is not preferred for various reasons. BTA is a very helpful agent to rescue postoperative over and undercorrections especially when used during postoperative early phase. Another indication of BTA is the augmentation of the effect of recession surgery peroperatively. BTA is not only used in adulthood strabismus but also in childhood strabismus.

Conclusion The cumulated experience of BTA treatment demonstrated that it is not just an alternative to surgery and it represents the most appropriate treatment modality in certain strabismus problems where surgery is not recommended for various reasons.

■ 3222

Botulinum toxin usage in extraocular muscle paralysis

YGGE J

Dept of Clinical Neuroscience, Karolinska Institutet, Stockholm

Purpose Botulinum toxin type A (BtxA) has proven to be an efficient treatment to reduce the long time outcome of restrictive esotropia after an acute sixth nerve paralysis. The BtxA when injected into the medial rectus muscle causes a temporary paralysis thus preventing its contracture and allows the antagonist (paralytic) lateral rectus muscle to take up the slack and reduce the ocular misalignment.

Methods In the present study patients with both traumatic as well as ischemic sixth nerve palsies were included. The patients had their paralysis onset between one and seven days before the initial investigation and treatment with an intramuscular injection of BtxA (usually 2.5 units of Botox[®], Allergan Inc.) into the medial rectus muscle under electromyographic monitoring. All patients had the paralytic esotropia measured using Maddox rod test before the treatment as well as at different time intervals after the treatment.

Results The results of the outcome will be discussed as well as an overview will be given of the results from other similar studies on acute sixth nerve paralysis.

Conclusion Botulinum toxin type A injections are useful in limiting the outcome of restrictive esotropia after an acute sixth nerve paralysis as well as minimizing the diplopia during recovery of such a paralysis.

■ 3223

The treatment for hemifacial and other essential spasms with botulinum toxin

PLANT G

Moorfields Eye Hospital, London

Purpose To describe the variety of clinical disorders in which occur involuntary movements of muscles innervated by the facial nerve.

Methods Clinical review and video demonstration.

Results Hemifacial spasm is common but is not the only condition which gives rise to involuntary spasms of facial muscles. Various manifestations of myokymia will be described, not all are idiopathic, but all can be treated with botulinum toxin. The need for imaging will also be discussed.

Conclusion Not all unilateral facial spasms are essential hemifacial spasm.

■ 3224

Other rare and useful indications of botulinum toxin

AYDIN P

-, Ankara

Purpose Amongst all other indication of botulinum toxin in ophthalmology, there are some rare clinical conditions where its usage is useful and helpful for the patient. Facial nerve paralysis with lagophthalmic keratitis and crocodile tears are amongst these rare indications. This presentation will include discussion of these rare and useful clinical situations. Interactive discussion will be encouraged following presentations.

■ 3241

Life and death decisions: the role of the IKK-JNK interplay

KARIN M

Department of Pharmacology, School of Medicine, University of California-San Diego, La Jolla, CA

Results The biological response to TNF α is determined by the balance between NF- κ B and JNK signaling, such that NF- κ B promotes survival, whereas JNK enhances cell death. Although the anti-apoptotic mechanisms triggered by NF- κ B are well established, the mechanism by which JNK promotes TNF α -induced death under conditions of reduced NF- κ B activity remained elusive. Here we show that JNK activation accelerates the turnover of the NF- κ B-induced anti-apoptotic protein c-FLIP. This, however, is not due to direct c-FLIP phosphorylation and instead depends on JNK-mediated activation of the E3 ubiquitin ligase Itch, which specifically ubiquitinates the long c-FLIP isoform and induces its proteasomal degradation. JNK1- or Itch-deficient mice or mice treated with a JNK inhibitor are resistant to three distinct models of acute liver failure and do not display inducible c-FLIPL ubiquitination and degradation, which are required for caspase-8 activation. Thus, JNK antagonizes NF- κ B during TNF α signaling by promoting the proteasomal elimination of c-FLIPL.

■ 3242

Promotion of lens epithelial to fiber differentiation by connexin 45.6, a role independent of gap junction communication

JIANG JX, BANKS EA, YU SX

Department of Biochemistry, University of Texas Health Science Center, San Antonio, TX

Results We have previously shown that one of the two chick lens fiber connexins, Cx45.6, plays a unique role in stimulating lens fiber differentiation and this function appears to be independent of gap junctions. In this study, we took advantage of previously identified natural loss-of-function mutations of Cx50 (D47A, E48K and P88S) and generated corresponding mutants of chick Cx45.6. Scrape loading and parachuting dye transfer analyses showed that all three Cx45.6 mutants failed to form functional gap junction channels. The degrees of cell differentiation were determined in primary lens cell cultures by either counting numbers of the "lentoid" structures, an indicator of lens fiber formation, or assessing the expression levels of lens differentiation markers major intrinsic protein (MIP) and δ -crystallin. Similar to the observation for wild type Cx45.6, overexpression of these Cx45.6 mutants achieved by recombinant retroviral infection increased cell differentiation shown by the enhanced expression of MIP and δ -crystallin. These results plus domain analysis reveal that lens epithelial-fiber differentiation relies on the expression of Cx45.6, but is independent of Cx45.6-mediated gap junction communication. The C-terminus of Cx45.6 is likely to play a predominant role in mediating this differentiation process.

■ 3243

G-proteins and their signaling pathways in eye development

LIU M, WENG J

Alkek Institute of Biosciences and Technology, and Department of Molecular and Cellular Medicine, Texas A&M University System Health Science Center, Houston, TX

Results Receptors coupled to heterotrimeric GTP-binding proteins (G-proteins) are integral membrane proteins involved in the transmission of signals from the extracellular environment to the cytoplasm. A variety of external stimuli, including hormones, neurotransmitter, phospholipids, and growth factors, can activate the G-protein coupled receptors (GPCRs), leading to the rapid activation of G-proteins and down-stream signaling pathways. My lab studies GPCR and Rho family of small GTPases in eye development and diseases. In this presentation, I will discuss a mouse model with a specific G-protein-coupled receptor deleted in which inactivation of the specific GPCR leads to a variety of defects, including small eyes, aniridia, and potential cataract in eye development.

■ 3244

Signaling transduction pathways mediating apoptosis in the ocular lens: the role of MAP kinases

LI DW (1, 2), LIU J (1)

(1) *Department of Biochemistry & Molecular Biology, University of Nebraska Medical Center, Omaha, Nebraska*(2) *Department of Ophthalmology & Visual Science, University of Nebraska Medical Center, Omaha, Nebraska*

Purpose Apoptosis of the lens epithelial cells plays an important role in mediating cataractogenesis. Different stress factors have been shown to induce apoptosis and also cause cataract. The signaling pathways mediating apoptosis and cataractogenesis, in most cases, remains unknown. The present communication will summarize the role of the mitogen activated kinases (MAPK) in mediating both apoptosis and cataractogenesis in the ocular lens.

Methods Various molecular and cellular biological methods have been used for the studies.

Results Upon induced by various stress conditions, three types of MAP kinases: ERK1/2, JNK1/2 and p38 kinase are activated and mediate apoptosis trigger by different stress factors.

Conclusion Although three types of MAP kinases can all mediate apoptosis, the ERK1/2 plays a key role in mediating stress-induced apoptosis in the ocular lens. The fact that ERK1/2 mediates stress-induced apoptosis in the ocular lens provides partial explanation that why the ocular lens never develops natural tumor.

■ 3251

The Basics of Shack-Hartmann Aberrometry

GICQUEL JJ, DIGHIERO PL

CHU Jean Bernard / Ophthalmology Department, Poitiers

Purpose Ophthalmological practice involves performing a full subjective refraction. The sphere, cylinder and axis of astigmatism are measured. Then we are only correcting two components of a whole host of refractive components of the optics of an eye. These two components (sphere and cylinder) constitute by far the majority of the optical aberration of an eye. Other monochromatic aberrations, also play a part as they increase with pupil size. In the majority of normal patients, these high order aberrations play a minor role, however, in cases of refractive surgery, keratoconus and orthokeratology, they can induce a number of visual disturbances. We will describe what higher-order aberrations are and how to measure them and give you a basic working knowledge of wavefront sensing (also known as aberrometry). We will show how wavefront sensors work, what are Zernike polynomials, what they tell us and we will show present and future clinical applications of wavefront aberrometry.

■ 3252

Custom Wavefront Soft Contact Lenses

LOPEZ-GIL N

Fisica, Universidad de Murcia, Murcia

Purpose The use of soft contact lenses (CLs) is the most practical and promoting way of correcting monochromatic aberrations. The presentation will show the potential limitations of this type of wavefront correction as well as optical and visual benefits after wearing customized CLs in subjects with different levels of high-order aberrations.

Methods Ocular wave-front aberration (OWA) of 4 normal eyes (myopic and astigmatic), 2 with keratoconus, and 2 after corneal transplantation (PK), were measured using a near infrared Shack-Hartmann sensor. OWA data obtained on each subject were used to simulate theoretical benefits of the wavefront correction by using soft contact lenses. We manufactured the personalized soft contact lens and compared the optical and visual performances of the eyes with these CLs and the standard correction (trial lenses).

Results Simulations in real eyes showed that the effectiveness of the correction depends on the particular wavefront, and optical and visual benefit of the pathological eye could be achieved despite typical rotations and translations of the CLs. However CLs translation could easily null the benefit of the high-order corrections in the normal eye. These simulations results are in agreement with the ones obtained experimentally after the subjects wore the customized CLs. In particular the keratoconic eyes with customized CLs reduced almost half of the high-order aberrations RMS for a 5 mm pupil, improving visual performances to normal levels (20/20).

Conclusion Both theoretical and experimental results show that low- and high-order aberrations could be corrected using soft contact lenses. Optical improvement respect to standard (second-order) correction, highly depends on contact lens final position and it is expected to be larger in pathological than in normal eyes.

■ 3253

Wavefront-based evaluation of functional optical zone after LASIK

NGUYEN KHOA JL (1), CHATEAU N (2), ASSOULINE M (3), LEBUISSON DA (1)

(1) -, Suresnes

(2) -, Orsay

(3) -, Paris

Purpose To use wavefront data to objectively estimate the functional optical zone (FOZ) in eyes treated with laser in situ keratomileusis (LASIK) for myopia. SETTING/ VENUE: Private practice

Methods Seventy-two eyes treated with LASIK using the Intralase FS2 15kHz femtosecond laser and the Bausch & Lomb 217z100 excimer laser with Zyoptix treatment software were retrospectively evaluated to determine the functional optical zone size. Three months postoperatively, FOZ was measured in wavefront aberrations maps by determining the largest pupil size that includes an area in which ray-tracing excluding defocus and astigmatism with varying pupil sizes is consistent with 20/20 vision. Preoperative and postoperative FOZ size was compared in each eye. Preoperative refraction, attempted correction, and achieved correction were correlated with the preoperative and postoperative FOZ using regression analysis.

Results After LASIK, the FOZ calculated using our method decreased a mean -1.57 ± 1.39 mm ($p < 0.001$). The size of the FOZ was correlated with high order aberrations RMS, attempted correction and achieved correction ($p < 0.05$). The FOZ reduction was significantly correlated with the postoperative HOA RMS variation, attempted correction, and achieved correction ($p < 0.05$). Before LASIK, there was no correlation between the FOZ and the preoperative manifest refraction ($p=NS$).

Conclusion The functional optical zone was reduced from the programmed ablation zone. Although higher attempted corrections had larger increases in spherical aberrations, larger treatment diameters minimized postoperative spherical aberrations. Larger optical zones provided less FOZ reduction.

■ 3254

Clinical Applications of Aberrometry

RADHAKRISHNAN H, O'DONNELL C

Faculty of Life Sciences, The University of Manchester, Manchester

Purpose Ocular aberrations have been a topic of interest to ophthalmic practitioners for over 200 years. The newfound clinical application of aberrometry in the field of refractive surgery has led to the development of several efficient methods of measuring ocular aberrations in a clinical setting. Apart from the major breakthrough that aberrometry has brought to the field of refractive surgery; it also proves worthwhile in several clinical applications which include: evaluating and diagnosing corneal degenerations, fitting contact lenses to presbyopes and patients with complex prescriptions, assessment of tear film especially in patients with dry eye, for measuring accommodative response curves and also for quantifying media opacities. We show that in such clinical conditions aberrometry can be used to provide additional clinical information which may prove helpful in diagnosis and/or management of the patient, although the standard polynomial fitting for aberrometry data may fail in some patients. Measurement of ocular aberrations provides an improved description of optical quality of the eye and hence can be useful to the clinician in evaluating eyes with optical abnormalities.

■ 3255**Optical quality of the eye after retropupillary fixation of the Artisan IOL**

DIGHIERO PL, GICQUEL JJ
CHU Jean Bernard / Ophthalmology Department, Poitiers

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

Methods Aphakic patients who had complicated phacoemulsification, leaving no capsular support, but good iris support and clear unwounded cornea were randomized for Artisan® (VerisyseTM) implantation site either implanted retropupilarly or over the iris. Wavefront aberrations were measured using the IRX3 Hartmann-Shack aberrometer at 4 mm pupil aperture diameter.

Results Best corrected visual acuity was significantly higher in the retropupilarly implanted group. There are no more wavefront aberrations with the retropupillary implantation technique of the Artisan® (VerisyseTM) than with the classic anterior one and obtained visual acuity is equal or better.

Conclusion Wavefront aberrometry helped us validate this new IOL implantation technique that is more respectful of the anatomy and may be considered as a good alternative to anterior chamber implantation in aphakic patients.

■ 3256**New developments in Aberrometry**

MARCOS S
CSIC, Instituto de Optica, Madrid

Purpose We will describe several laboratory-based aberrometers and will discuss advances in the interpretation and analysis of ocular aberration data (i.e. how do ocular aberrations depend on wavelength? how important is sampling density?). We will present new understanding of optical aberrations in normal human eyes as well as several clinical applications in the fields of refractive surgery, cataract surgery and contact lenses, from a scientific perspective.

■ 3257**Our experience on aberrometry in refractive surgery**

MONTES MICO R
~, Alicante

ABSTRACT NOT PROVIDED**■ 3258****An adaptive optics device for the simulation of visual outcomes**

CHATEAU N (1), VABRE L (2), NGUYEN KHOA JL (3)
(1) Imagine Eyes, Orsay
(2) Observatoire de Meudon, Meudon
(3) Hôpital Foch, Suresnes

Purpose To explore the possibilities of a new adaptive optics device, used for the correction and generation of ocular aberrations, as well as for the study of visual performance.

Methods An adaptive optics device, combining a high-dynamic deformable mirror, a high-precision wavefront aberrometer, and a versatile stimulation system was built. It provides procedures for measuring, correcting, generating aberrations. A set of aberrated artificial eyes was used to assess the capacity of the system to measure and correct ocular wavefront aberrations. Dynamic wavefront correction (closed-loop) was then performed on real eyes, and the RMS quality of the correction was measured. The system was finally used to explore visual outcomes after manipulating aberrations: changes in visual acuity after wavefront correction, and in the presence of user-defined aberrations.

Results Highly accurate generation and correction of wavefront aberrations were measured, over a wide dynamic range: 4D of astigmatism in a 6mm pupil, 3rd order +/- 10µm PV, 4th order +/- 6µm PV, with a difference (measured – predicted) of 0.008µm in average. Closed-loop correction on real eyes was achieved with a residual RMS error between 0.04 and 0.1µm over a 6 mm pupil. First visual acuity tests with aberrations correction showed a significant gain with the correction of the higher-order wavefront.

Conclusion The dynamic range and precision of the adaptive-optics system allows its use in a wide variety of eyes. It provides a clinical-compatible tool able to precisely simulate changes in ocular aberrations. It may help for a variety of applications where predicting visual outcomes with aberrations is needed.

■ 3261

Femtosecond laser (FSL) surgery and scanning microscopy in branch retinal vein occlusion (BRVO): experimental pilot study

TOROPYGIN SG (1), KRALISE MHJ (2), RIEMANN I (3), HILD M (2),
 KHURIEVA EL (2), LOEW U (2), MESTRES P (4), SEITZ B (2), RUPRECHT KW (2),
 KOENIG K (3)

(1) Department of Ophthalmology, Tver State Medical Academy, Tver
 (2) Department of Ophthalmology and Eye Hospital, University of Saarland, Homburg
 (3) Fraunhofer Institute for Biomedical Engineering, St. Ingbert
 (4) Electron Microscopy Unit, Department of Anatomy and Cell Biology, Saarland University, Homburg

Purpose Non-amplified FSL pulses were applied in pilot study of in vitro ablation and multiphoton scanning microscopy (MSM) in models of vascular occlusion.

Methods Lumens of the main retinal veins of seven porcine eyes were irradiated with a 90 MHz near-infrared 750-nm Ti:Sa laser (power: 1 W, pulse width: <140 fs). Intravascular line-pattern ablation was performed within 10/0 monofilament synthetic nylon suture material (3 retinas) and within human hairs (4 retinas) inserted into the vascular lumens. Laser treated (7 retinas) and control (2 retinas) specimens were subjected to laser MSM, light microscopy and electron microscopy.

Results Precise FSL cuts within suture material and hairs were achieved in all specimens. Laser exposition time and pulse power determined width of the lesions which ranged from 1 to 10 µm. Neither laser MSM nor histology revealed collateral damage of the vascular wall.

Conclusion Non-amplified near infrared FSL pulses at low ($nJ\cdot\mu$) pulse energies may be a new promising strategy for in vivo precise non-contact ablation of intravenous material without morphologically detected collateral damage of the venous walls. High resolution laser MSM offers 3D non-invasive non-destructive imaging at submicrometer resolution within seconds before and after FSL ablation. The technique allows to predefine targets and to observe the progression of laser tissue removal. Supported by the Alexander von Humboldt Foundation, Bonn, Germany.

■ 3263

Local RPE cells migrate on the uneven surface of two potential Bruch's membrane substitutes after subretinal implantation

MOLNAR FE (1, 2), YELLACHICH D (1), LENG T (1), HUIIE P (1), DALAL R (1), MARMOR MF (1), FISHMAN HA (1), BLUMENKRANZ MS (1)

(1) Ophthalmology, Stanford University, Stanford
 (2) Universitat-Augenklinik, Freiburg

Purpose The need for replacement of aged Bruch's membrane is a major limitation to subretinal pigment epithelium (PE) transplantation. Artificial Bruch's membrane substitutes are possible solutions. We explored the use of polycarbonate (PC) and cellulose ester (CE) membranes, and compared the behavior of the membranes after subretinal implantation into the rabbit eye.

Methods CE with 100kD molecular weight cut off and original thickness of 100-micron were ablated with a 193-nm excimer laser to create 30 and 10-micron thick membranes. PC membrane with a pore size of 0.4µm was commercially available as 10-micron thick material. Both membrane types were implanted into the subretinal space of New Zealand red rabbits. Eyes were removed between the 1st and 8th week after implantation, and histological changes were analyzed by light microscopy and transmission electron microscopy (TEM).

Results Both CE and PC membranes were well-tolerated in the subretinal space of rabbits. Light microscopy shows that RPE cells migrated onto both membrane types and that the photoreceptor outer segments were well-preserved where RPE migration occurred. TEM sections showed attached RPE cells on the uneven surface of the membranes.

Conclusion CE and PC membranes are two new substrates that could serve as artificial Bruch's membrane substitutes. PC and CE membranes can be implanted subretinally without rejection, although PC is easier to manipulate. Their uneven surface characteristics help the adhesion of PE cells, and the membrane-RPE complexes in vivo supported photoreceptor outer segment preservation.

■ 3262

Multiphoton Imaging and Disruption of the Retina

HEISTERKAMP A (1), BALIMGART J (1), KRUEGER RR (2), LUBATSCHOWSKI H (1)
 (1) Laser Zentrum Hannover, Hannover
 (2) Cole Eye Institute, Cleveland

Purpose The purpose of this work was to demonstrate the possibility of combined multiphoton imaging and cutting with micrometer precision at the retina in ex vivo pig eyes by ultrashort laser pulses.

Methods Using a femtosecond, titanium-sapphire laser system working at wavelengths between 700 and 950 nm we used the autofluorescence and second harmonic signals induced by the laser at the retina to acquire an microscopic image of the retina. At higher laser intensities, the disruption of the tissue was enabled and used to cut retina with sub micrometer precision. A water immersion objective was used to focus the laser radiation into the retina, while the laser was scanned over the retina. The fluorescence and SHG signals were collected using a photomultiplier.

Results Multiphoton autofluorescence was useful in identifying cellular structures of the different domains and layers of the retina. Moreover, very precise cutting of the retina was enabled, showing no collateral damage of the surrounding structures.

Conclusion Multiphoton autofluorescence and SHG microscopy have been found to be an appropriate technique for resolving the cellular structures of retina of ex vivo porcine eyes. Moreover, the application of ultrashort laser pulses for high resolution manipulation of the retina is feasible.

■ 3264

Prospective randomized clinical trial comparing face down position and semi seated position after surgery for idiopathic full-thickness macular hole: preliminary results.

GARCHER CREUZOT C (1), GUILLAUMEY A (1), LAFONTAINE PO (1), MALVITTE L (1), HUBERT I (2), BRON AM (1), BERROD JP (2)

(1) Department of Ophthalmology, Dijon
 (2) Department of Ophthalmology, Nancy

Purpose To determine the influence of face down position on anatomic and functional results after macular hole (MH) surgery.

Methods Patients suffering from macular holes (stages 2 to 4) were enrolled in this study. Visual acuity was measured before and three months after surgery. Macular OCT was performed before and three months after surgery to measure the diameter of macular holes and to assess the postoperative status. Pars plana vitrectomy, inner limiting membrane peeling and fluid-air exchange were performed. A non expanding mixture of air and SF6 and air and C2F6 was used for pneumatic tamponade in MH less than 500 µm and larger than 500 µm respectively. Eyes were randomized in two groups: P0 and P1. Patients in group P0 were asked to keep a semi-seated position 10 hours per day for five days. Patients in group P1 were asked to keep a face down position 10 hours per day for five days. Minimum follow up was three months.

Results One hundred and four eyes were enrolled. In the P0 group, closure of the MH at three months occurred in 45/50 eyes (90%). In the P1 group, closure of the MH occurred in 52/54 eyes (96%). For MH < 500 µm the success rate was 30/32 eyes in group P0 (94%) and 37/38 eyes in group P1 (97%). The difference between groups was not significant. The median of visual acuity increased in each group without a statistically significant difference between groups.

Conclusion The position after macular hole surgery does not seem to have a strong influence on the outcome of the surgery, especially for small size macular holes.

■ 3265**VEGF and PEDF in early PVR; analysis using the IntraOcular Fluid Bank Maastricht**

DIEUDONNE SC (1), LA HEIJ EC (1), DIEDEREN R (1, 2), KESSELS A (3),

LIEM A (1), KIJLSTRA A (1), HENDRIKSE F (1)

(1) *Ophthalmology, Maastricht*(2) *Basic Neuroscience, Maastricht*(3) *Clinical Epidemiology and Medical Technology Assessment, Maastricht*

Purpose In proliferative diabetic retinopathy (PDR) the PEDF/VEGF balance is disturbed probably resulting in neovascularization and fibrosis. An imbalance was also found in non-vascularized proliferative vitreoretinopathy (PVR). That study concerned end-stage PVR. For early PVR pathogenesis, the PEDF/VEGF balance is unknown and its analysis was the purpose of our study.

Methods To study early PVR, subretinal fluids were routinely collected at the time of primary retinal detachment surgery and stored in our IOB Bank. Herein, we found 29 patients who developed PVR after surgery. Their subretinal PEDF/VEGF concentrations were compared with those of 90 PVR-negative patients. Since most studies analyze vitreous, we verified our base-line laboratory results using vitreous samples from 14 patients with macular hole/pucker. Furthermore, as positive controls for PEDF/VEGF imbalance we analyzed vitreous from nine PDR eyes.

Results PEDF and VEGF concentrations were not different between PVR-positive or -negative patients. Jointly, their (median) PEDF concentration was much higher (9.6 µg/ml) than base-line vitreous (0.33 µg/ml; p<0.001) or PDR vitreous (0.36 µg/ml). Their VEGF concentration was also higher (0.071 ng/ml) than base-line vitreous (0.008 ng/ml), but lower than PDR vitreous (0.877 ng/ml). Remarkably, when we regrouped the patients into diabetics – they had no clinical signs of PDR - and non-diabetics, PEDF levels were higher for the diabetic subgroup (P = 0.003).

Conclusion The balance between VEGF and PEDF in eyes with retinal detachment is shifted in favor of angiogenesis control, when compared to eyes with PDR. This may explain the absence of neovascularization following retinal detachment.

■ 3266 / 302**Internal limiting membrane peeling during macular hole surgery: a comparison of outcomes with and without indocyanine green and trypan blue assistance**

LAMBLEY RG, BATRA R, EWINGS P, GRAY R

Taunton and Somerset Hospital NHS Trust, Taunton

Purpose To compare anatomical and visual outcomes of macular hole surgery using indocyanine green (ICG), trypan blue (TB) or using no stain to visualise the internal limiting membrane (ILM).

Methods Retrospective analysis of 50 consecutive cases of idiopathic macular hole undergoing pars plana vitrectomy and ILM peel with or without ICG or TB assistance. ICG was used in 12 eyes, TB in 24, and the remaining 14 eyes underwent ILM peel without a stain. Where stains were used, 0.1ml of 0.5% ICG or 0.1ml of 0.15% TB was injected over the macula and left for one minute before aspiration. Outcome measures were anatomical hole closure and change in Snellen visual acuity (VA).

Results The mean number of Snellen lines of improvement in visual acuity was greater in the no-stain group (3.71 lines) than in the ICG group (2.33 lines) and the TB group (2.63 lines; p=0.07). 7/14 (50.0%) of the no-stain group had a final VA of 6/12 or better compared with 5/12 (41.7%) in the ICG group and 12/24 (50.0%) in the TB group. Anatomical hole closure was achieved in 10/12 eyes (83.3%) in the ICG group, 23/24 (95.8%) eyes in the TB group, and 12/14 (85.7%) eyes in the no-stain group.

Conclusion ILM peeling without a stain to help visualization was associated with higher number of lines of improvement on Snellen acuity testing than ICG and TB-assisted peeling, and with similar anatomical success. Previous clinical studies have linked poor surgical outcomes with ICG toxicity; our results indicate a similar toxic effect of TB.

■ 3267 / 303**TEM of epiretinal tissue in diabetic macular oedema**

SYNEK S (1), PAC L (2), SYNKOVA M (1)

(1) *Ophthalmology and Optometry, Medical Faculty, Masaryk University, Brno*(2) *Anatomy, Medical Faculty, Masaryk University, Brno*

Purpose Diabetic cystoid macular oedema (DME) is common cause of visual acuity decrease. Good anatomical results and visual acuity(VA) of pars plana vitrectomy(PPV) in a case of macular hole with internal limiting membrane peeling leads to usage of this technique in DME. Favourable results even in a case without vitreoretinal traction leads to conclusion that pathogenesis of this disease is different.

Methods 21eyes from 19 patients with DME that had undergone PPV and peeling ILM were analyzed retrospectively. Half of them were laser treated before surgery. All eyes had an attached posterior hyaloid's membrane in the macular region, but without thickening and without traction.Parts of excised tissues were examined by transmissive electron microscopy(TEM).

Results Median duration of DME at the time of PPV was approximately 11.0 months. The median preoperative best-corrected VA of 0.08, improved to a median postoperative VA of 0.25. 7 eyes without preoperative laser coagulation had a median VA improvement of 77%, while 12 eyes with preoperative macular laser treatment had a median VA improvement of 14.8%. In all 21 eyes, DME was no longer visible on microscopic examination after a median period of 3.0 months after PPV. TEM samples contained ILM, glial cells and connective tissue and can be classified in monolayer membrane, multilayer membrane and true epimacular fibrous membrane.

Conclusion PPV and peeling ILM resulted in the resolution of oedema, with an improvement in visual acuity in the majority of cases. Eyes without preoperative macular photoocoagulation had a significantly higher visual improvement than eyes with preoperative laser treatment. A randomized controlled prospective trial of PPV versus laser is needed to determine the role of PPV as treatment modality for DME

■ 3311

Pathophysiology of mitochondrial optic neuropathies: are LHON and DOA a variation on the same theme?

CARELLI V
-, Bologna

ABSTRACT NOT PROVIDED

Abstract not provided

■ 3313

Mitochondrial division proteins in *C. elegans* and mammals

VAN DER BLIEK A
Department of Biological Chemistry, UCLA School of Medicine, Los Angeles

Purpose Although a small but growing number of mitochondrial fusion and division proteins are known, it has also become clear that large gaps in our knowledge of mitochondrial membrane dynamics still exist. In the hope of filling some of these gaps, we are using a variety of classical and reverse genetic approaches in *C. elegans* to search for novel factors that regulate the structure of mitochondria and their cristae. An update on these searches will be given. In addition, we are investigating the function of *C. elegans* MGM-1 and its human homologue Opa1. These proteins are localized to the mitochondrial intermembrane space where they are required for fusion between mitochondria. A *C. elegans* mutant called eat-3 is mutated in the chromosomal MGM-1 gene. As in other organisms, mutations in *C. elegans* MGM-1 cause excessive mitochondrial fragmentation. Electron microscopy of eat-3 mutants shows frequently occurring septae within mitochondria, suggestive of a block in inner membrane fusion. Electron tomography shows a reduction in the size and numbers of cristae (collaboration with Carmen Mannella, Wadsworth NY), indicating a role in maintaining normal mitochondrial structure along with the well-established role in mitochondrial fusion. The mammalian homologue, Opa1, is proteolytically cleaved and released into the cytosol during apoptosis. The mitochondria of apoptotic cells become fragmented similar to the fragmentation observed with Opa1 siRNA. The opposite effect is observed with siRNA of the mitochondrial intermembrane space protease Yme1. Yme1 siRNA prevents constitutive cleavage of Opa1 and it gives rise to connected mitochondria. We conclude that Yme1 constitutively regulates Opa1, while apoptosis and loss of membrane potential induce further proteolysis, inactivating Opa1 and causing its release from membrane.

■ 3312

Genetic dissection of OPA1 functions in mitochondrial fusion and apoptosis

SCORRANO L
Dulbecco-Telethon Institute, Venetian Institute of Molecular Medicine, Padova

Purpose Optic Atrophy 1 (OPA1) is a dynamin related protein of the inner mitochondrial membrane, whose mutations lead to dominant optic atrophy. OPA1 belongs to a growing family of proteins that regulate mitochondrial shape during life and death of the cell. Mitochondria amplify activation of caspases during apoptosis by releasing cytochrome c and other cofactors. This is accompanied by fragmentation of the organelle and remodeling of the cristae. Molecular mechanisms governing the latter remain unclear.

Methods We investigated the role of OPA1 in apoptosis and mitochondrial fusion combining genetics, physiology, imaging and biochemistry.

Results OPA1 protects from apoptosis by preventing cytochrome c release. This is independent from mitochondrial fusion but depends on the oligomerization of two forms of OPA1, the soluble, intermembrane space and the inner membrane integral one. The pro-apoptotic BCL-2 family member BID disrupts OPA1 oligomers, while high levels of OPA1 stabilize them and prevent mobilization of cytochrome c. OPA1 does not interfere with activation of the mitochondrial "gatekeepers" BAX and BAK, but controls shape of mitochondrial cristae during apoptosis. This is dependent on the inner mitochondrial membrane rhomboid protease PRL, which is required to generate the intermembrane space form of OPA1.

Conclusion Thus, OPA1 has genetically and molecularly distinct functions in mitochondrial fusion and in cristae remodelling during apoptosis.

■ 3314

A model of OPA1 optic atrophy: protein-truncating nonsense mutation in mouse opa1 GTPase

VOTRUBA M (1, 2), PIECHOTA MJ (1), HOLLINS AJ (1), DAVIES VJ (1)
(1) *School of Optometry & Vision Sciences, Cardiff University, Cardiff*
(2) *Cardiff Eye Unit, University Hospital Wales, Cardiff*

Purpose Heterozygous mutations in the OPA1 gene, which lead to haploinsufficiency, cause dominant optic atrophy (ADOA). Visual deficit results from loss of retinal ganglion cells (RGCs). We have generated a mouse model of human ADOA in order to study the pathophysiology of ADOA.

Methods An ENU mutagenised C3H DNA sperm archive was screened for opa1 mutations. A heterozygous mutation in exon 8 coding for a C to T transition at 1051bp was detected, which is predicted to result in protein truncation (Gln 285 to Stop: Q285X) at the start of the central GTPase protein domain. IVF with C57Bl/6 generated F1 hybrids. PCR excluding rd1 (pdeB) and opa1 allele-specific genotyping, were used to direct breeding with C57Bl/6. The consequences of haploinsufficiency on opa1 protein production were examined.

Results By F4 no opa1^{-/-} animals have been detected, and opa1^{-/-} appears to be embryonic lethal before E9. Western blot analysis shows a ca. 50% reduction in opa1 protein in retina. At 6 months there is a reduction in rabbit anti-mouse anti-opa1 C-terminal polyclonal antibody immunostaining observed in retinal sections, specifically in the RGC layer. However, at 6 months of age dilated fundus examination did not reveal any observable pathology in opa1^{+/-} mice, and there were comparable RGC counts on H&E sections in opa1^{+/-} and WT controls.

Conclusion Opal^{-/-} embryonic lethality suggests a vital early role for opa1 and confirms that the GTPase and dynamin-central regions are of key importance to protein function. The subtle abnormalities detected at 6 months of age, in the presence of reduced opa1 protein production, highlight the need to age this model and assess the detrimental effects of various stressors, such as light.

■ 3315

OPA1, from functions in mitochondria to dysfunctions in optic neuropathies

LENAERS G (1), DELETTRE C (1), CORNILLE K (1), SOUKKARIEH C (1),
CHEN-KLIO-CHANG M (1), ELACHOURY G (1), DEPEYRE C (1), ARNDT C (1),
PAYET O (1), MULLER A (1), BONNEAU D (2), AMATI-BONNEAU P (2),
REYNIER P (2), OLICHON A (3), BELENGUER P (4), HAMEL CP (1)

(1) Institut des Neurosciences de Montpellier, Montpellier

(2) INSERM U694, Laboratoire de Biochimie et Biologie Moléculaire, Angers

(3) EMBL, Heidelberg

(4) LBCMCP, UMR5088 du CNRS, Toulouse

Purpose Dominant optic neuropathies include the dominant optic atrophy (DOA) and other rare syndromic clinical presentations. Two genes (OPA1 and OPA3) and 2 loci (OPA4 and OPA5) have been identified in DOA. Both genes, like those responsible for the Leber hereditary optic neuropathy (LHON), encode mitochondrial proteins, indicating that mitochondria are key elements in the pathogenesis of inherited optic neuropathies.

Methods Since many OPA1 patients have truncating mutations that lead to loss-of-function alleles, we performed siRNA-mediated silencing in HeLa and retinal ganglion cells. Clinical investigations were performed on 7 affected OPA1 patients and matched controls.

Results We found in HeLa cells, that OPA1 alternate splicing defines functions in mitochondrial network fusion and apoptosis, uncoupling both events. In RGC cultures, OPA1 functions are similar to those in other cell types. On the other hand, intravitreal injection of OPA1 siRNA leads to apoptosis of RGCs in pups, and to a reversible decrease of VEPs in adult mice, while ERG were unaffected. MRI spectroscopy in severely affected OPA1 patients detects lactate accumulation in the occipital region. Further abnormal distribution of mitochondrial activities was found in muscle biopsies, although respiration parameters did not evidence deficiencies.

Conclusion Altogether, these results suggest that OPA1 controls the mitochondrial network structure, the capacity to respond to the energetic demand and eventually triggers apoptosis. These findings will be compared to those found in LHON and other DOA to postulate common mechanisms of mitochondrial impairment in RGCs.

■ 3321

Autofluorescence Lifetime Imaging of the Human Fundus

SCHWEITZER D, SCHWEITZER F, HAMMER M

Experimental Ophthalmology, University of Jena, Jena

Purpose Metabolic changes are assumed as first pathological signs of fundus diseases. In such early state, alterations are reversible. To estimate the consumption of oxygen, the oxygen saturation should be measured in arteries and veins. The metabolic state at cellular level can be estimated by comparing of autofluorescence of several endogenous fluorophores.

Methods Utilising absorption spectra of reduced and oxygenated haemoglobin, the oxygen saturation of blood can be determined by means of imaging spectrometry. The 2-dimensional distribution of the optical density of macular pigment xanthophylls can be determined from blue images, taken prior fluorescence angiography. The discrimination of fluorophores can be realised according to specific fluorescence lifetime after excitation by picosecond laser pulses.

Results In F1-generation of AMD –patients, both the consumption of oxygen and the optical density of xanthophylls is increased in comparison with healthy subjects. In fluorescence lifetime images of normals, the shortest lifetime (e.g. 140 ps) was detected in the macula, whereas the optic disc exhibits much longer values. In AMD, fluorescence lifetimes are much longer at all fundus sites and the macula exhibits not more the shortest lifetime.

Conclusion The increased consumption of oxygen in children of AMD patients point to metabolic processes, actively increasing the optical density of xanthophylls. Lifetime measurements in connection with selective excitation and emission measurements have the potential for estimation of metabolic state in mitochondria.

■ 3322

Clinical applications of autofluorescence imaging: review of pathology and indications

PETO T (1), DANDEKAR SS (2), JENKINS S (2), BIRD AC (2)

(1) Reading Centre, London

(2) Moorfields Eye Hospital, London

Purpose Autofluorescence imaging has been developed to evaluate the integrity and health of the outer retina in ocular diseases. Autofluorescence is derived from lipofuscin in the Retinal Pigment Epithelium (RPE) cells. Increased autofluorescence usually demonstrates excessive accumulation of lipofuscin and precedes photoreceptor degeneration. Decreased autofluorescence signals loss of photoreceptors with or without RPE loss.

Methods In a clinical setting changes in patterns of autofluorescence are noted and together with the clinical setting they might be unique enough to establish diagnosis, understand prognosis and provide patients with appropriate information about their disease. We will concentrate on three areas during this talk, age related macular degeneration (AMD), retinal dystrophies (RD) and Mitochondrially Inherited Diabetes and Deafness (MIDD).

Results In AMD, there can be discrepancies between clinical and angiographic findings and visual acuity. In these cases autofluorescence imaging is the key factor to ascertain the integrity of the outer retina and as such provide guidance on prognosis. In RD, autofluorescence imaging is pivotal to establish the diagnosis and for follow-up of the patients, especially in cases where there is unexplained visual loss. The unique pattern of decreased patchy autofluorescence in MIDD is diagnostic in many cases and helps identification of affected individuals without using invasive techniques.

Conclusion Autofluorescence imaging is becoming a well-utilised technique in clinical setting, as it is easy to use and the information obtained is invariably helpful to both clinicians and patients.

■ 3323

Non-Invasive In Vivo Measurement of Retinal Physiology using High-Speed, Ultrahigh Resolution Optical Coherence Tomography

SRINIVASAN VJ (1), WOJTKOWSKI MD (1, 2), LIU JJ (1), DUKER JS (2), CLERMONT A (3), BURSELL SE (3), FUJIMOTO JG (1)

(1) Dept. of Electrical Engineering & Computer Science and the Research Laboratory of Electronics, Massachusetts Institute of Technology, Cambridge, MA

(2) Tufts-New England Medical Center, New England Eye Center, Boston, MA

(3) Research Division and Beetham Eye Institute, Joslin Diabetes Center, Departments of Ophthalmology and Medicine, Harvard Medical School, Boston, MA

Purpose Previous studies demonstrated optical coherence tomography (OCT) for the measurement of ex vivo functional changes in isolated neurons and excised retinal preparations, as well as in vivo functional changes in the cerebral cortex. We investigate in vivo imaging of retinal function in the rat model and propose methods for similar measurements in un-anesthetized human subjects.

Methods Measurements are performed using a high-speed, ultrahigh resolution OCT system. A transient white light stimulus is delivered to the retinas of healthy, anesthetized rats and the OCT reflectance is recorded as a function of time. The spectrum of wavelengths used in the OCT measurement is well outside the sensitivity range of the rat retina.

Results The functional recording showed that a white light stimulus induced a 10-15% increase in the average amplitude reflectance from the photoreceptor outer segments. Appropriate control experiments confirmed the physiologic nature of the response.

Conclusion In vivo functional OCT is demonstrated in the rat retina, and may have potential as a non-invasive, objective technique for measuring retinal function. To our knowledge, this is the first demonstration of functional OCT in the intact retina, and may be an important step toward developing this technology for human studies.

■ 3324

Using Nanoshells to achieve molecular contrast in OCT : study results and review of other contrast agents for OCT

FABER DJ (1, 2), DE BRUIN DM (1), VAN VELTHOVEN ME (3), NOISSER T (4), GRAFC (4), ECKART R (4), VERBRAAK FD (1, 2), VAN LEEUWEN TG (1, 5)

(1) Laser Center AMC, Amsterdam

(2) Ophthalmology AMC, Amsterdam

(3) AMC, University of Amsterdam, Amsterdam

(4) Institut für Physikalische Chemie, Universität Würzburg, Würzburg

(5) Biophysical Engineering, Biomedical Technology Institute, University of Twente, Twente

Purpose Nanoparticles play an increasingly important role in medical research, e.g. as drug delivery vehicles and contrast agents. Bio-functionalization of the nanoparticles allows targeting to specific cells or molecules of interest. This enables of a novel class of high resolution imaging methods, dubbed nanoparticle-assisted optical molecular imaging (NAOMI). We propose to use contrast agents based on gold-silica nanoshells, nanoparticles with tunable optical properties.

Methods We used Optical Coherence Tomography to visualize nanoshells, optimized for our detection wavelengths (800 nm) injected in an ex vivo porcine eye.

Results The nanoshells showed up as bright reflecting structures in the OCT images

Conclusion The results show the potential of NAOMI for high resolution, quantitative molecular imaging.

■ 3325

Progress in the flying spot dual imaging en-face OCT/confocal channel instrument

PODOLEANU A (1), CUCUR (1), BRADUA (1), DOBRE GM (1), ROSEN R (2), GARCIA P (2), ROGERS JA (3), HATHAWAY M (3), PEDRO J (3), WEISS R (3), VAN VELTHOVEN ME (4), DE SMET M (4), ROSA CC (5), MERINO D (6), DAINTY C (6)

(1) *Applied Optics Group, University of Kent, Canterbury*

(2) *New York Eye And Ear Infirmary, New York*

(3) *Ophthalmic Technologies Inc., Toronto*

(4) *Amsterdam Medical Centre, Amsterdam*

(5) *University of Porto, Porto*

(6) *National University of Ireland, Galway*

Purpose To report different imaging possibilities opened by the en-face flying spot OCT concept.

Methods We researched, designed, assembled and tested imaging instruments for the eye which have at least two channels. They assemble images from T-scans (which are transverse profiles of reflectivity). In some implementations we added aberration correction via an Adaptive Optics (AO) closed loop.

Results We have designed multiple channel imaging systems such as: OCT/SLO, OCT/OCT, OCT/angiography, OCT/SLO/angiography, OCT/AO and OCT/SLO/AO. These channels can work simultaneously or sequentially. All the versions mentioned are versatile in respect to their regime of operation, as they can perform A-scanning, B-scanning and C-scanning, such possibility being allowed by using T-scans (transverse profiles of reflectivity) to construct the images.

Conclusion En-face OCT imaging opens new ways for high resolution imaging of the eye.

■ 3326

Proteomics in Eye Diseases

MISSOTTEN GS

LUMC, Leiden

Purpose In recent years, molecular genetics have greatly advanced our understanding of a lot of ocular diseases. However, as a way to understand disease mechanisms better, it can not always give us a clear answer: are structural gene mutations actually expressed, and have promoter and regulatory gene mutations a real effect on protein synthesis. Additionally, the function of many genes is not known as is the identity of many proteins they code for. Since Noble Price winner Takana introduced his matrix enhanced mass spectrometry (MALDI) and Fenn the electrospray ionisation (ESI) in the late eighties, proteomics tries to give the answers that genetics could not give us.

Methods By analysing the results of different papers on proteomics in ocular diseases, and our own experience in uveal melanoma eyes, the different techniques and possibilities of proteomics are explored.

Conclusion This paper tries to emphasize the impact of proteome analysis on future ophthalmology.

■ 3331

Uveal melanoma: Epidemiologic profile and treatment in an Ocular Oncology unit of the Hospital Clinico Universitario de Valladolid

SAORNIL MA (1, 2), GARCIA-ALVAREZ C (1, 2), ALMARAZA A (3), MUÑOZ MF (3), LOPEZ-LARA F (4), DE FRUTOS-BARAJA JM (4), MUÑOS Y (1, 2)

(1) *Ophthalmology*, Hospital Clinico Universitario, Valladolid

(2) *IOBA*, Valladolid

(3) *Epidemiology*, Hospital Clinico Universitario, Valladolid

(4) *Radiotherapy*, Hospital Clinico Universitario, Valladolid

Purpose To analyze the epidemiological features and treatment of uveal melanoma patients diagnosed in the ocular oncology unit at the Hospital Clínico Universitario de Valladolid between 1992 and 2006

Methods 232 consecutive patients were evaluated. Demographic data, clinical ocular and systemic features and treatment were registered in a data base. Numeric variables have been analysed by average and standard deviation and quantitative or categorical variables by frequency tables.

Results Mean age was 61.31y (SD=14.8), 129 were male (55.6%) and 103 female (44.4%); 166 (71.9%) were symptomatic and 58 (25.1%) were diagnosed in a routine examination. Visual acuity in the affected eye was < 0.1 in 67 (29.1%), 0.1 to 0.5 in 65 (28.3%), 0.5 to 0.8 in 30 (13.04) and > 0.8 in 68 (29.6%). Tumour was infiltrative in 25 cases (10.8%), nodular in 142 (61.2%) and mushroom-shape in 65 (28%). Tumour size was small in 38 (16.5%), medium in 123 (53.2%), and large in 70 (30.3%). In 177 cases (77%) signs of tumour activity were found. Extraocular extension was present in 15 patients (6.6%) and signs of systemic disease in 2 (0.9%). Initial treatment was primary enucleation in 63 patients (27%), episcleral brachytherapy in 102 (43%), Proton beam radiotherapy in 8 (3.5%), surgical resection 3 (1.3%) and observation 46 (19.8%).

Conclusion In the present series most uveal melanomas are diagnosed when they became symptomatic and have a medium-large size, leading to a high rate of enucleated eyes. Early detection would be desirable to increase the rate of patients eligible for conservative treatmentPartially supported by grant Jose M^a Aguilar Bartolomé

■ 3333

Transpupillary thermotherapy of choroidal melanoma: a prospective analysis

PARROZZANI R (1), MIDENA E (1, 2), BOCCASSINI B (1), VUJOSEVIC S (2)

(1) *Department of Ophthalmology*, University of Padova, Padova

(2) *Fondazione GB Bietti per l'Oftalmologia*, IRCCS, Roma

Purpose To evaluate clinical outcomes of transpupillary thermotherapy (TTT) as primary treatment of small choroidal melanoma.

Methods Seventy-seven patients affected by choroidal melanoma were treated with TTT using infrared diode laser (810 nm), with a beam diameter of 2-3 mm. Patients were followed every 6 months with full ophthalmological and systemic examination.

Results Mean tumor thickness was 2.0 +/- 0.7 mm, and mean tumor largest basal diameter was 6.0 +/- 2.4 mm. Mean follow-up was 55.2 +/- 17.9 months. Mean visual acuity change after TTT was -1.9 +/- 3.2 EDTRS lines. After TTT tumor regressed in 70 eyes (90.9 %). Seven eyes (9.1 %) showed tumor recurrence; five were successfully re-treated with Iodine 125 brachytherapy and two eyes were enucleated. Ophthalmic complications were found in 20 patients (26.0 %), and included: macular pucker (14.3 %), branch retinal vascular occlusion (7.8 %), macular oedema (3.9 %), neovascular glaucoma (3.9 %), vitreous and sub-retinal haemorrhage (2.6 %). Two patients (2.6 %) developed liver metastasis and died during follow-up.

Conclusion Transpupillary thermotherapy, with a close monitoring of early and late local complications and recurrences, may be a clinically effective method for conservative treatment of selected small posterior choroidal melanomas.

■ 3332

Choroidal perfusion in eyes with untreated choroidal melanoma

RESCH H (1), GARHOFER G (1, 2), SCHMETTERER L (1, 3), ZEHETMAYER M (2), DORNER GT (2)

(1) *Department of Clinical Pharmacology*, Vienna

(2) *Department of Ophthalmology*, Vienna

(3) *Department of Biomedical Engineering and Physics*, Vienna

Purpose Previous evidence suggests that ocular blood flow is altered in eyes with choroidal melanoma. In the present study we assessed pulsatile choroidal blood flow (POBF) with two different techniques and investigated whether there exists a correlation between tumor size and ocular blood flow parameters.

Methods 22 patients with unilateral untreated choroidal melanoma were studied in an assessor masked study using two methods: POBF was measured with a commercially available pneumotonometer and local foveal fundus pulsation amplitude (FPA) by laser interferometry. Results as obtained with these techniques were correlated. In addition, the tumor height was measured with standardized A-scan echography and the tumor's basal dimensions with B-scan echography. Tumor volume was calculated using the ellipsoidal solid model and was correlated to POBF and FPA.

Results There was no significant difference in intraocular pressure between affected and contralateral eyes. POBF tended to be higher in tumor eyes compared to unaffected eyes (1061 +/- 326 µl/min vs. 922 +/- 344 µl/min; p = 0.2). FPAs also tended to be higher in affected eyes but again the difference did not reach the level of significance (3.8 +/- 0.8 µm vs. 3.6 +/- 0.8 µm; p = 0.5). Both methods showed a high degree of correlation in unaffected eyes ($r = 0.90$, $p < 0.001$). POBF and FPA were also correlated in tumor eyes, but the correlation was slightly less pronounced ($r = 0.85$, $p < 0.001$). There was no correlation between tumor volume (306 +/- 252 mm³) and POBF or FPA.

Conclusion In conclusion our results do not indicate choroidal hyperperfusion in patients with choroidal melanoma. Accordingly, the clinical usefulness of measuring POBF in tumor patients may be limited.

■ 3334

Uveal metastasis and ocular hypertony

POLIRNARAS JAC, SCHALENBOURG A, ZOGRAFOS L

Jules Gonin Eye Hospital, Lausanne

Purpose To investigate the presence of ocular hypertony among patients presenting uveal metastasis

Methods Using a computerized database, we analyzed 318 patients presenting uveal metastasis from 1960 to 2004. A complete ophthalmological examination was performed, including the visual acuity, the inflammatory activity of the anterior chamber, the intraocular pressure, the fundus examination with photography, an echography and, if needed, an UBM. The origin of the primary tumor was assessed. Furthermore, the characteristics of the ocular metastasis, their treatment modality and the survival analysis were tabulated.

Results Among 318 patients presenting uveal metastasis, we reported 21 cases (6.6%) associated with ocular hypertony including 15 women (4.7%) and 6 men (1.9%). The primary tumors were breast carcinoma (n:11), cutaneous melanoma (n:4), pulmonary neoplasia (n:2), systemic melanoma (n:1), larynx carcinoma (n:1), stomach cancer (n:1) and unknown origin (n:1). The following mechanisms, associated or alone, were involved in the development of ocular hypertony: the tumoral invasion of the iridociliary angle (n:11; 52.4%), the trabecular blockade by anterior displacement of the iridociliary complex (n:7; 33.3%) and the rubeosis iridis (n:4; 19.1%). In Kaplan-Meier survival curves, an increase of the mortality among the patients with ocular hypertony was observed. Following irradiation treatment, eye retention probability was 97.2%.

Conclusion Depending of the localization, the extension and the specific mechanism, an ocular hypertony could install in eyes affected by uveal metastasis by the reviewed mechanisms.

■ 3335

Potential Blood Markers for the Detection of Metastatic Uveal Melanoma

BARAK V (1), FRENKEL S (2), FOLBERG R (3), MAJUMDAR D (4),
KALICKMAN I (1), PEER J (2)

(1) Oncology, Hadassah-Hebrew University Medical Center, Jerusalem
(2) Ophthalmology, Hadassah-Hebrew University Medical Center, Jerusalem
(3) Pathology, University of Illinois, Chicago, IL
(4) Math Statistics and Computer Science, University of Illinois, Chicago, IL

Purpose To evaluate the possible role of three molecules - Osteopontin, S-100 β and MIA - as biomarkers for early detection of metastatic uveal melanoma.

Methods We tested the blood serum of 38 patients treated for uveal melanoma who survived disease-free for at least 10 years, 18 patients with metastatic uveal melanoma (8 of these patients were diagnosed with metastasis during follow-up), and 44 age-matched controls without a history of malignant disease. Serum levels of Osteopontin (ng/ml) (R&D Systems), S-100 β (μ g/L) (DiaSorin, Inc.), and MIA (ng/ml) (Roche) were detected by ELISA. Statistical analysis included t-test, sign test (nonparametric), analysis of variance (ANOVA) and ROC analysis.

Results A statistically significant difference between high Osteopontin, ($p=0.0037$), MIA ($p=0.0005$) and S-100 β ($p=0.0111$) levels in patients with metastatic uveal melanoma and low levels in disease-free (DF) patients and controls was demonstrated. Significant differences were found between the levels of all 3 markers in the metastatic stage of the 8 patients, as compared to their pre-metastatic stage ($p<0.05$). ROC analysis was performed for metastatic patients vs. controls and vs. disease-free patients and revealed AUC for single tests: MIA - 88%, S-100 β - 77%, OPN - 73% and for combinations: OPN+S100 - 85%, OPN+MIA - 82%, and for all 3 markers - 91%.

Conclusion Serum Osteopontin, MIA and S-100 β levels distinguish well between metastatic and DF patients, and therefore will be useful markers for early detection of metastasis in uveal melanoma patients. Increases in serum Osteopontin, MIA and S-100 β levels may indicate the development of metastatic uveal melanoma.

■ 3336

Trends in treatment and prognostic factors for conjunctival melanoma in a tertiary referral ocular oncology service over the last 50 years

MALIK ANJ (1, 2), HUNGERFORD JL (1, 2)

(1) Moorfields Eye Hospital, London
(2) St Bartholomew's Hospital, London

Purpose Identify and compare treatment and prognostic factors for recurrence and tumour related mortality of conjunctival melanoma in the last 50 years.

Methods Retrospective analysis of clinical and histopathological data of 54 patients from 1995-2005 and comparison with 256 patients from a previous study in the same unit (from 1948-1994).

Results Risk factors for recurrence of disease on univariate analysis were 'unfavourable' location of tumour (hazard ratio= 4.44, $p=0.01$) and multifocality (hazard ratio= 3.26, $p=0.032$). Treatment with excision alone occurred in 20% ($n=11$) of patients, compared with 57.4 % in the previous study. 31.5% ($n=17$) received more than one adjuvant treatment (cryotherapy, radiotherapy, mitomycin c in any combination), compared with 24% previously. There was no significant difference in recurrence rates of disease or tumour related mortality rates between treatment with excision alone or excision and adjuvant treatment. Overall there was a 1% risk (4/54) of exenteration (primary and secondary) per person years follow-up compared to a 4% risk (95/256) in the previously studied cohort. Risk of local recurrent disease was 6.2% per person years compared to 6.0% in the previous cohort. Kaplan Meier estimate of 5 year mortality was 13.8%, in contrast with 17.1%.

Conclusion Prognostic factors remain similar in both studies. There is a strong trend towards the increasing use of adjuvant treatments, while treatment with exenteration has declined. Rates of recurrent disease and tumour related mortality do not show any significant change. The exact benefit of adjuvant treatments in mortality and recurrence of disease remains difficult to establish with retrospective analysis.

■ 3341**Rationale for use of capsular tension rings - a review of forces acting on the lens capsular bag***LIU C**Sussex Eye Hospital, Brighton*

Purpose To review the forces acting on the lens capsular bag both per-operatively and post-operatively.

Methods Systematic theoretical analysis of such forces, and how they could be counteracted.

Results The forces acting on the lens capsular bag include the following: Trampoline effect during collapse and re-inflation of the anterior chamber. Capsulorrhesis exerts forces on the lens capsule as well as zonules. The same applies to the phaco probe and second instrument. Aspiration of soft lens matter can exert tension on zonules if the anterior capsule is engaged. Insertion of intraocular lens and dialling it to position can stretch the anterior lens capsule and exert forces on the zonules. Long term contraction of the capsular bag from lens epithelial cell transdifferentiation occurs especially in cases of zonular weakness, inadequately sized anterior capsulorrhesis, and silicone IOL material. Gentle tissue handling is important especially in cases of zonular weakness. Long term bag contraction can be reduced by adequate size of anterior capsular opening and choice of IOL material. The lens capsular bag can be supported per-operatively with iris retraction hooks and the Mackool Cataract Support System. The Ahmed Capsular Tension Segment can provide sectorial capsular support both per- and post-operatively. Single or multiple capsular tension rings prevent capsular contraction. The Cionni modified capsular tension ring prevent capsular bag decentration.

Conclusion Knowledge of forces acting on the capsular bag and how to counteract them reduces the risk of operative complications during and after cataract surgery, especially in high risk cases.

■ 3342**The pros and cons of a one-piece ring-IOL***GALAND A**Cataract and Intraocular Implant Clinic, Rotheux-Rimière, Liège*

Purpose Capsular rings may have different goals: 1. To give tension in order to stabilize the bag. 2. To avoid capsular folds. 3. To impede lens cells migration as a mechanical barrier. 4. To delivery intraocular drugs. 5. To be part of a multi-pieces implant ("gigogne")

Methods I have experience of capsular rings and of a one-piece ring IOL implant. I have placed around 20 capsular rings and around 120 one-piece ring IOL. We did a review of charts and of videos of these cases.

Results The pros of one-piece are easier insertion and better haptic-optic relationship. This configuration do allow some degree of accommodation. The cons of one-piece are difficult manufacturing and risk of implant damage when injected through a cartridge.

Conclusion A one-piece ring IOL implant has definite clinical advantages

■ 3343**Spring constants and foldable equator rings***KURZ S (1), DICK HB (2)**(1) University of Mainz, Mainz**(2) University of Bochum, Bochum-Langendreer*

Purpose We compared the elastic boundaries of rigid capsular tension rings (CTR) by determining their spring constant. Furthermore, a foldable capsular equator ring system with sharp edge design is demonstrated.

Methods 14 different types made of PMMA provided by 9 companies were tested. The CTRs were positioned in a special retainer of a material testing machine and were compressed by a constant speed of 10 mm/min. The spring constant of each CTR was tested three times. The closed foldable capsular ring consists of a mixture of 8 hydrophobic and 8 hydrophilic ring segments. Because of its flexibility it can be implanted through various kinds of cartridge systems as well as using a forceps. The ring was implanted in more than 200 human eyes after phacoemulsification.

Results All tested rigid CTRs showed a linear proportionality between force and compression distance. The CTRs of each type showed comparable mechanical properties. The CTRs demonstrated greater differences in spring constants, ranging from 0.8162 mN/mm in mean to 4.5501 mN/mm. After implantation of the capsular equator ring, no postoperative complications like capsular folds or inward-bending were observed after a 6 months follow-up. Posterior capsular opacification was minimal or absent in all eyes.

Conclusion The spring constant of a CTR represents a suitable mechanical characteristic facilitation the choice of a certain CTR model depending on the individual situation. A CTR with a low spring constant may be advantageous for the management of zonulolysis, while a CTR with a great spring constant might inhibit capsular bag shrinkage more effectively. In contrast to rigid capsular tension rings the foldable equator ring formed a predesigned capsular bag size and helped reducing zonular stress during implantation.

■ 3344**Surgical technique and complications of CTRs***LITTLE BC**Royal Free Hospital, London*

Purpose Capsular Tension Rings (CTR) have significantly improved the safety and outcomes for phacoemulsification in the presence of compromised zonules. Although they have been available in Europe since 1993 (Morcher GmbH) they only recently gained FDA approval for use in the USA. They are an instantaneously appealing concept and easily understood in principle but there are plenty of pitfalls in practice and some unique complications that are associated with their use. This is especially true for the inexperienced surgeon and occasional user. They can be used either electively ("cold": planned in eyes that have had previous trauma or lens dislocation/instability) or unplanned ("hot": intraoperative zonular dehiscence). I have implanted over 40 of these devices: some "cold" but mostly "hot" and I have discussed my experiences with many colleagues and by doing so learned a great deal. I have tried to condense this experience into my presentation in order to offer you not only some very practical tips and tricks for the reliable and safe insertion of CTRs but also to demonstrate how things can go wrong, how to avoid these problems and what to do when they happen.

■ 3351

Amniotic membrane-covered bioprostheses for treatment of ocular surface disease

CURSIEFFEN C, KRUISE FE

Dept. of Ophthalmology, University of Erlangen-Nürnberg, Erlangen

Purpose Amniotic membrane transplantation is an established method for treatment of ocular surface disease. Here we demonstrate a new non-contact method by implantation of amniotic-membrane covered bioprostheses for treatment of ocular surface defects.

Methods Ildig-devices (n=2) were covered by sheets of amniotic membrane on the operating table outside the patients eye (nylon 10-0 sutures, cryofixed amniotic membrane, epithelial layer outwards). In topical anaesthesia, these prostheses were then placed onto the eyes of patients where a suture-fixed amniotic membrane transplantation was undesirable: persistent erosion secondary to chronic graft-versus-host-disease (n=1) and oculus ultimus with persistent corneal erosion due to neurotrophic keratopathy after keratoplasty (n=1). Implants were left in place for two weeks and then removed. Corneal epithelial healing and condition of amniotic membrane were examined (routine histology).

Results Amniotic membrane-covered bioprostheses were implanted and explanted without problems. Epithelial healing occurred within two weeks after implantation in these two pilot patients. Histological work-up revealed an intact amniotic membrane epithelium.

Conclusion Amniotic membrane-covered bioprostheses seem to be a feasible approach for „non-contact“ transplantation of amniotic membrane onto the ocular surface in patients where suture fixation is not desirable or where problems with anaesthesia prevent suturing of the ocular surface. Future studies will have to analyze, whether the greater amount of amniotic membrane tissue brought onto the ocular surface positively affects the speed of wound healing.

■ 3352

In vivo confocal microscopy in limbal epithelial damageNUBILE M, LANZINI M, LIBERALI T, CALIENNO R, MASTROPASQUA L
Ophthalmic Clinic - University G. d'Annunzio Chieti-Pescara, Chieti

Purpose To evaluate in vivo confocal microscopic (IVCM) pattern of limbal damage related to chemical burns or severe inflammation of the ocular surface.

Methods IVCM was used to evaluate limbal epithelial morphology in 15 patients (Group 1) affected either by alkali burn (n=7) or by severe inflammation of the ocular surface due to chronic corneal ulcers (n=8), and in 30 healthy control subjects (Group 2). IVCM was performed over 360°. Limbal epithelial cell density was calculated by using dedicated analysis software. IVCM wide field reconstructed images of limbal area were compared to impression cytology (IC) at the same area. Follow-up extended up to 6 months.

Results Limbal epithelial morphology of the healthy controls (Group 2) was characterized by continuous epithelial cells which showed radial infolding corresponding to the Vogt's palisades and crypts. Mean superficial epithelial cell density in Group 2 was 1545 ± 355 cells/mm². The morphology of the superficial epithelial cell layer extending from the conjunctiva to the peripheral cornea showed a pattern of transition which well compared with IC. In 33% of cases (n=3 chemical burns; n=2 ulcers) belonging to Group 1, marked disruption of the limbal epithelial morphology, extending at least for 6 clock hours, characterized by loss of transition pattern and radial infolding, markedly reduced epithelial cell density (421 ± 266 cells/mm²), and severe signs of inflammation was observed. Those cases developed partial or total limbal stem cell deficiency (LSCD) during the follow-up. 66% of cases of Group 1 showed less severe limbal lesions and progressively underwent anatomical recovery.

Conclusion Microscopic signs of limbal epithelial cell layer damage may early predict clinical evolution to LSCD

■ 3353

Neurotrophic regeneration during the corneal wound healing after refractive surgery

BLANCO-MEZQUITA JT (1), MERAYO-LLOVES JM (1)

MARTINEZ-GARCIA MC (2), BONINIS (3), LAMBIASE A (3), PROENCA R (4)

(1) Instituto Universitario De Oftalmobiología Aplicada, Valladolid

(2) Cell Biology, Valladolid

(3) Laboratory of Ophthalmology, Rome

(4) IBILI, Coimbra

Purpose The cornea is one of the most densely innervated tissues of the body, which is highly abounded by sensory nerves and autonomic nerve fibers. Corneal nerves fibers exert important trophic influences on the corneal epithelium and contributes to maintain the cornea and promote wound healing after external injuries. The aim of this work is to evaluate the neurotrophic role of efferent axons in the corneal wound healing process after refractive surgery in hens.

Methods Lohmann classic 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. Clinical monitoring was made during two months. Eyes were enucleated at sequential time points and fixed in 10% buffered formalin. Sections were stained with H-E and Masson tricromic.Occludin, Claudin-5, Hsp-70, α -SMA, Trk-A and Gap-43 expression was studied by immunohistochemistry.

Results Statistically significant differences in the grade of haze and pachymetry measurements were found. There were not differences in the expression of Occludin and Claudin-5 in the corneal epithelium, among groups.A significant increase in Hsp-70, α -SMA and Trk-A expression was observed in the NGF group. Statistically significant differences were observed in the expression of GAP-43 between groups. Gap-43 expression was minor in the NGF group.

Conclusion Topical NGF modify trophic axon regeneration after PRK surgery and can be used to modulate the corneal wound healing process.

■ 3354

Accelerated corneal wound healing in Cx43+/- mice

GICQUEL JJ (1), MILIN S (2), LEVILLAIN PM (2), MESNIL M (3), DIGHIERO PL (1)

(1) Department of Ophthalmology, University Hospital, Poitiers

(2) Department of Pathology, University Hospital, Poitiers

(3) Gap Junctions Team, CNRS-UMR 6558, University of Sciences, Poitiers

Purpose To compare the corneal wound healing rate after acute ocular alkali burns between swiss wild type and connexin 43 deficient mice (Cx43+/-)

Methods A chemical burn with 2 microlitre of sodium hydroxide 0.15 mol/L was created in one eye of 25 mice.The epithelial defect was photographed and measured every day for 15 days.Ocular burn damage was assessed. On day 15, eyes of each mouse were enucleated and analyzed histopathologically.

Results Healing of a corneal epithelial injury in Cx43+/- mice was significantly faster compared with Cx43+/+ mice. Histopathologically, the epithelium encountered after alkali burn in the Cx43+/- group was of equal quality or better compared to the Cx43+/+ group.

Conclusion These observations indicate that Cx43 expressed in injured epithelium may modulate cell behavior such as adhesion or migration, thus contributing to corneal epithelial wound healing. Targetting Cx43 expression in wounded corneas may help achieve faster healing.

■ 3355 / 340

NGF promotes the healing of bilateral recurrent corneal erosion in dogs

DODI PL (1), COSTA N (2), ALOE L (3)

(1) Dept. of Animal Health - University of Parma, Parma

(2) Dept. of Pharmacy Sciences - University of "Magna Grecia", Catanzaro

(3) Inst. of Neurobiology and Molecular Medicine - CNR EBRI, Rome

Purpose To report a case of bilateral recurrent corneal erosion treated with Nerve Growth Factor.

Methods A 6-year-old female French Bulldog with a bilateral recurrent ulcer covering over 80% of the right eye surface and 40% of the left one. Several treatments were tried over a 5 weeks period without any improvement. Before NGF treatment cell debris were removed using a dry cotton swab to encourage epithelial attachment; the corneal erosion was topically treated with purified murine NGF diluted in paraffin oil (value: 50 microgram/ml) for 4 weeks (2 drops every 12 hours).

Results Four days after NGF treatment the ulcers showed neurovascolorization with size reduction. After 2 weeks the cornea was negative to fluorescein, the central leucoma was vascularized. A further 3 weeks review showed the leucoma was slightly evident; at the end of the therapy the cornea showed a significant improvement of transparency.

Conclusion This report showed that bilateral recurrent corneal erosion in a French Bulldog was successfully healed with topical application of NGF. This result confirmed and extended previous evidences on human cornea's ulcers. NGF caused also a marked anti-inflammatory action of the dog's cornea and a "remodelling" action of the corneal epithelium. The present findings suggest a therapeutic potentiality of NGF, particularly when other available therapies fail to promote healing.

■ 3356 / 341

Towards the Development of Purpose-designed Ocular Bandage Lenses

BATEMAN S, MANN A, TIGHE BJ

Biomaterials Research Unit, Aston University, Birmingham

Purpose This poster is concerned with recognized analogies between the cornea & chronic wound sites as the basis for rational materials design of bandage lenses for ocular wounds.

Methods Macromer technology has enabled a common synthetic base to be used for fabrication of hydrogel-based contact lens materials & wound dressings. Nelfilcon functionalised PVA (Ciba Vision) used in conjunction with purpose-designed macromers enables the fixed charge density & functional group balance to be approximately equated to wound tissue. The materials are applied to the bed of chronic slow/non-healing ulcerated wounds, wound fluid is sampled for analysis & the state of healing of the wound noted. Removed dressings are retained for analysis. A similar nelfilcon macromer base is used as the basis for fabrication of ocular bandage lenses.

Results Initial clinical results show enhanced healing when there is ionic similarity of hydrogel surface & wound tissue. Microlitre-scale assays for a series of biochemical markers have been established and are used to monitor progress of wounds. Similar markers are found in the contact lens-wearing eye and, importantly, some (eg vitronectin) are predominantly associated with the posterior surface, and others (eg kininogen) with the anterior surface. The elimination or down-regulation of certain biochemical moieties (eg bradykinin), coupled with the normal accumulation of other factors (eg vitronectin) are clearly important to enhance ocular healing.

Conclusion Parallel studies on wound dressing & contact lens materials & the biochemistry of these two body sites are providing useful insight into the design of materials for ocular bandage lenses which provide a more effective healing environment than current cosmetic contact lenses.

■ 3357 / 342

Early limbal stem cell deficiency in KID Syndrome

LANGMAN ME (1, 2), GICQUEL JJ (3), MILIN S (4), LEVILLAIN PM (4), MESNIL M (5), DIGHIERO PL (3)

(1) St Louis Hospital / Ophthalmology Department, St Jean d'Angély

(2) Jean Bernard University Hospital / Ophthalmology Department, Poitiers

(3) Ophthalmology / Jean Bernard University Hospital, Poitiers

(4) Pathology / Jean Bernard University Hospital, Poitiers

(5) UFR Sciences Fondamentales et Appliquées / Physiology, Poitiers

Purpose To present one case of early limbal stem cell deficiency in a seven year-old boy with authentified KID syndrome (isolated mutation D50N of the GJB2 gene).

Methods The boy was referred to our department by his dermatologist for systematic ophthalmological examination. Refraction, slit lamp examination, tear film study with fluorescein, maebomian secretions examination and impression cytology (IC) were performed. Two IC specimens were collected for each eye; one for Nelson classification, the other for the study of the expression of HLA-DR in flow cytometry.

Results Slit lamp examination revealed a calm white eye. The cornea limited peripheral stromal invation on 360° in both eyes with no preponderance for contact areas with the eyelids. Break up time was normal OS. No maebomian dysfunction was found. Nelson score confirmed limbal stem cell deficiency while flow cytometry showed no overexpression of HLA-DR compared with data obtained from normal subjects.

Conclusion Limbal stem cell deficiency can be found in young patients with KID syndrome. It seems not due to chronic ocular surface inflammation nor maebomian dysfunction but to primary limbal stem cell dysfunction caused by the mutation of the connexin 26 gene.

■ 3361

Autofluorescence findings in Acute Exudative Polymorphous Vitelliform Maculopathy

VACLAVIK V (1), BIRD AC (1, 2), ROBSON AG (3), OOI KGJ (4), HOLDER GE (3), WEBSTER AR (2, 1)

(1) Moorfields Eye Hospital, London

(2) Institut of Ophthalmology, London

(3) Electrophysiology MEH, London

(4) Institut of Ophthalmology UCL, London

Purpose Acute Idiopathic Exudative Polymorphous Vitelliform Maculopathy (AIEPVM), is a rare disorder characterized by multifocal exudative yellow-white posterior pole lesions. All previously described patients had acute onset of headaches followed by visual loss associated with vitelliform lesions and bilateral serous detachments. The autofluorescence (AF) findings of 3 AIEPVM patients are described.

Methods Three patients aged 38, 35 and 48 years presented with sudden loss of central vision, following a flu-like illness with headaches. All three patients were in good health with no family history for any eye condition. They had a complete ophthalmic examination, color photos, AF imaging, OCT, ISCEV-standard ERG and EOG. One also had fluorescein angiography.

Results At presentation, all patients had reduced visual acuity (VA) and multiple yellow-white lesions at the level of the retinal pigment epithelium (RPE) with serous detachment. AF images showed hyperautofluorescent lesions corresponding to the deposits. The ERG was normal in all patients and the EOG, performed 12-18 months after the onset, was normal in 2 cases, borderline in 1. Several weeks later, all patients had improved the VA, which became normal within 1 year. The hyperautofluorescent lesions reduced in size and gravitated.

Conclusion The concept that yellow deposits in AIEPVM contain lipofuscin is substantiated by the AF imaging in our cases. The RPE dysfunction initiated by stimuli (inflam., immune-mediated) might explain the lipofuscin overload. In convalescent AIEPVM the RPE regain ability to phagocytose the deposits as evidenced by spontaneous clinical resolution and decrease in AF. AF imaging is helpful in diagnosis and monitoring of clinical course of AIEPVM.

■ 3363

Laser phototherapy for von Hippel disease - two case studies

VIDE ESCADA EICKMANN A (1), HENRIQUES J (1), NASCIMENTO J (1), CASTANHEIRA-DINIS A (2)

(1) Retina Department - Instituto de Oftalmologia Dr. Gama Pinto, Lisbon

(2) Instituto de Oftalmologia Dr. Gama Pinto, Lisbon

Purpose To describe the clinical evaluation of angiomas retinae in 2 patients. A review of the current literature is undertaken, focusing on the available therapeutic options.

Methods Two patients, one male, 27 years old and one female, 26 years old both presented with loss of vision due to the presence of an unilateral retinal hemangioma (RH), as confirmed by fluorescein angiography (FA). Both underwent several sessions of laser photocoagulation therapy with decrease in activity of tumour following each session. Both patients underwent periodic examinations for clinical signs and symptoms of von Hippel-Lindau disease. Mean follow-up was 5.7 years.

Results Stabilization of RH was achieved, although, with poor final visual acuity (10/200 and 5/125) due to associated macular changes. The female patient presented a successfully managed lesion with laser photocoagulation around and intra the tumour. No signs or symptoms of von Hippel-Lindau disease have yet been observed.

Conclusion In von Hippel disease therapeutic efficacy and visual acuity preservation is dependent on the early detection and treatment of lesions. We hypothesise that indirect laser photothermic therapy in a PRP pattern associated to direct photocoagulation of the lesion might prove to be more effective than phototherapy of the feeder vessels. We expect that the use of anti-VEGF drugs might provide a stronger and better contribution to the resolution of RH and so to the preservation of visual function. We recommend that all patients should undergo periodic testing for signs of von Hippel-Lindau disease.

■ 3362

Age dependence of macular changes in congenital achromatopsia

VARSANYI B, LESCH B, SOMFAI GM, FARKAS A

Semmelweis University Eye Clinic, Budapest

Purpose Congenital achromatopsia is a retinal disorder based on the lack of functioning cone photoreceptors. Previous morphological results showed a remarkable structural change and significantly lower retinal thickness in patients with achromatopsia. Aim of this study was to model the possible time course of the disorder by comparison of the morphology of younger and older patients.

Methods Optical coherence tomography (OCT) was performed in eight patients with congenital achromatopsia and eight age matched healthy controls. The results of the younger (N=5; mean age: 10yrs, ±2,8) and older (N=3; mean age: 56,3yrs, ±15) patients was compared. Retinal thickness (RT) was measured by automated (ETDRS regions, retinal thickness) and manual (A-scans, callipers) methods. Total macular volume (TMV) was also calculated by the built in software of the OCT device.

Results Significant lowering was found in the TMV of the "older" compared to that of the "younger" patients with achromatopsia. Retinal thickness was also lower in the older patients than the younger ones. This thinning was significant in the parafoveal macula, but not in the foveola. There was no difference in the retinal thickness or the TMV between the younger and older healthy control subjects.

Conclusion Achromatopsia is a congenital, stationery disorder; the cones are not functioning (or absent) from birth. The lower values of retinal thickness measured in the older patients is most probably due to age related degeneration of some cone-related structures, but not the cone photoreceptors themselves. This theory seems to be supported by the fact, that the thinning of the retina was more prominent in the parafoveal area than in the fovea.

■ 3364

Bothnia Dystrophy, a genetical, clinical and electrophysiological study

BIRSTEDT MSI, SANDGREN O

Clinical Sciences, Ophthalmology, Umeå

Purpose To describe the unique phenotype Bothnia Dystrophy (BD). Nightblindness, progressive maculopathy and fundus changes with retinitis punctata albescens changes are present in this phenotype. The prevalence (1:3600) found in Västerbotten County, Sweden gives us an opportunity to further investigate these patients.

Methods Electrophysiological studies to evaluate the extremely prolonged dark adaptation in BD and a battery of objective tests of visual function were made understand the phenotype in BD patients.

Results The mutated carrier-protein (CRALBP), localized in the RPE, and Müller cells, tightens the retinoid binding properties in the visual cycle. A compromised rod function, dysfunction of the Müller cells, disturbed function of the inner retina and grossly affected RPE, were found. With prolonged dark adaptation (10 and 24h), an increase in retinal sensitivity to light and improvement of the ERG occurred indicating extremely prolonged synthesis of photopigments, retardation of the visual process in the RPE and loss of retinal cells probably starting early in BD. Indications that the retinal Müller cells may be involved in the pathogenesis of BD were also found.

Conclusion The possibility that recycling of retinoids localized in the RPE might be impaired in BD might give future therapeutic possibilities. Due to the large and clinically well-characterized set of patients with this disease, they constitute a suitable study group.

■ 3365**Retinal vascular changes in patients with impaired glucose tolerance and early diabetes**

SUMMANEN PA (1), VON WENDT G (2), LINDSTRÖM J (3), PELTONEN M (3),
UUSITIUPA M (4), TUOMILEHTO J (3)

(1) Ophthalmology, Helsinki University Central Hospital, Helsinki

(2) Ophthalmology, Sankt Eriks Ögonsjukhus, Stockholm

(3) National Public Health Institute, Helsinki

(4) Public Health and Clinical Nutrition, Kuopio

Purpose To assess retinal vascular changes in persons with impaired glucose tolerance (IGT).

Methods A cross-sectional study with 251 persons with IGT participating in the Diabetes Prevention Study (DPS) (mean age 54 years (SD 7, range 39–68), 130 in the intervention group. Macula centered 45 degree red-free black-and-white 12 x 12 cm prints were graded in a masked fashion for retinopathy (RP) and arteriolosclerotic changes. At least one eye was gradable in all but 4.

Results Vascular changes were common: focal arteriolar narrowing (n=34), decreased arteriovenous ratio (n=200), increased wall reflex (n=206), elongation (n=89) or straightening (n=5), arteriovenous nicking (AVN) (n=181), venous wall irregularity (n=200), dilation (n=157) and tortuosity (n=36). Patients with focal arteriolar narrowing had significantly higher systolic blood pressure (142 vs. 133 mmHg, P=0.01) and those with at least one AVN higher triglycerides (1.6 vs. 1.4 mmol/l, P=0.019) than those without. 45 patients had RP, 24 in the control and 21 in the intervention group (20.5% vs. 16%). Persons without RP tended to have lower serum triglycerides (1.6 vs. 1.8 mmol/l, P=0.079) and systolic (133 vs. 140 mmHg, P=0.03) but not diastolic blood pressure; age, weight, body mass index, total cholesterol, HDL or fasting glucose value or 2 hour glucose tolerance test were similar. By the time of photography, 78 persons (31%) had diabetes, 33 in the intervention and 45 in the control group (25% vs. 37%).

Conclusion Arteriolosclerotic changes were common and RP detected in one fifth of the persons with IGT or early diabetes. Systolic blood pressure was associated with focal arteriolar narrowing and RP, dyslipidemia with AVN.

■ 3366**Identification of different phenotypes of nonproliferative retinopathy of diabetes type 2 using cluster and discriminant analysis**

NUNES S (1), BERNARDES R (1), DUARTE L (1), CLINHA-VAZ J (1, 2)

(1) AIBILI-Association for Innovation and Biomedical Research on Light and Image, Coimbra

(2) Department of Ophthalmology, Coimbra University Hospital, Centre of Ophthalmology, IBILI – Institute of Biomedical Research on Light and Image, Faculty of Medicine, University of Coimbra, Coimbra

Purpose To test the existence of different phenotypes of nonproliferative diabetic retinopathy (NPDR) progression in type 2 diabetic patients using mathematical segmentation techniques.

Methods Thirty-five eyes from 35 patients with type 2 diabetes, under stabilized metabolic control and with mild NPDR, were followed up for 2 years with systemic and ophthalmic examinations at 6-month intervals. The ophthalmologic measurements obtained from field-2 of the fundus were microaneurysms (MA), and levels of retinal leakage (RL), retinal thickness (RT), and blood flow (BF). Each repeated measurement counted as an independent variable, allowing for data segmentation by cluster analysis to identify phenotypes of NPDR progression. Ward's hierarchical clustering method was first used to explore the number of clusters underlying the sample. Second, k-means clustering characterized the k selected clusters. Reproducibility and stability of the clustering solutions were analyzed by discriminant analysis and changes in cluster membership, respectively.

Results The k=3 solution achieved a stability of 80.0% and reproducibility of 97.7% by k-means clustering, based on the accumulated number of MA and levels of RL, RT and BF. Based on this solution, phenotype 1 was characterized by low ophthalmic parameter values, i.e., low RL, RT, number of accumulated MA, and BF. Phenotype 2 was characterized predominantly by high RL, and phenotype 3 by a high number of accumulated MA.

Conclusion Cluster analysis identified 3 different phenotypes in 35 type 2 diabetic patients under stabilized metabolic control and with mild NPDR, for a 2-year period, with high reproducibility and stability.

■ 3411

Genotype-phenotype correlations in mitochondrial optic neuropathies

AMATI-BONNEAU P, FERRE M, VERNY C, MALTHIER Y, BONNEAU D,
REYNIER P
INSERM U694, University Hospital, Angers

Purpose Mitochondrial optic neuropathies include Autosomal dominant optic atrophy (ADOA), Leber's hereditary optic neuropathy (LHON) and autosomal dominant optic atrophy and cataract (ADOAC). ADOA generally starts in childhood and is characterized by a progressive bilateral decrease in visual acuity, dyschromatopsia and optic nerve pallor. OPA1 gene is responsible for 40% of the cases of ADOA. OPA1 encodes a dynamin-related GTPase which controls the balance of mitochondrial fusion and fission. LHON is characterized by bilateral optic atrophy responsible for acute or subacute visual loss, usually starting between the ages of 18 and 35. In addition to optic atrophy, some patients present neurological signs of central nervous system involvement. LHON is caused by specific mutations in mitochondrial DNA. ADOAC is due to mutations in OPA3 gene which encodes for a mitochondrial protein.

Methods We report here a molecular study of 600 patients with optic atrophy referred to our center during the period 2001-2005. Of these, 60% were familial and 40% sporadic cases.

Results Pathogenic mutations were identified in 290 patients (48%). Mitochondrial DNA mutations were found in 90 patients (15%), OPA1 mutations in 186 patients (31%) and OPA3 mutations in 14 patients (2%). A specific genotype-phenotype correlation was observed in patients harboring the R445H OPA1 mutation and optic atrophy associated to deafness. About 10% of sporadic patients carried pathogenic mutation in OPA1.

Conclusion Mitochondrial DNA and OPA1-OPA3 gene analyses allow diagnosing about 50% of the patients. Our results suggest that patients could greatly benefit from neurological investigations and that sporadic cases need to be explored at the molecular level.

■ 3412

Biochemical & molecular analysis of skin fibroblasts from patients with mitochondrial optic neuropathy

REYNIER P, CHEVROLLIER A, VERNY C, LOISEAU D, POUJOL MA, GUILLET V,
MALTHIER Y, AMATI-BONNEAU P, BONNEAU D
Inserm U694, Angers

Purpose Hereditary optic atrophies are heterogeneous genetic disorders due to the degenerescence of retinal ganglion cells and optic nerve fibers. Autosomal dominant optic atrophy due to OPA1 gene mutations and Leber's hereditary optic neuropathy due to mitochondrial DNA mutations are the commonest forms. Other rarer forms of hereditary optic atrophy include X-linked and autosomal recessive optic atrophies. Finally, more than 15 disorders have combined optic atrophy and extra-ocular anomalies such as Costeff syndrome due to OPA3 gene mutations and a form of axonal neuropathy with optic atrophy caused by MFN2 gene mutations. All known genes involved in these pathologies code for ubiquitous mitochondrial proteins and altered mitochondrial functions are likely to play an essential role in the pathogenesis.

Methods We have collected the skin fibroblasts from 20 patients affected by various forms of hereditary optic neuropathies and from 10 controls. Biochemical investigations were performed in order to explore the energetic metabolism in these cells.

Results In all these pathologies, a mitochondrial coupling impairment and a decrease of the membrane potential was observed. An ATP production defect was also observed in the fibroblasts of patients carrying the R445H OPA1 mutation.

Conclusion These results reinforce the hypothesis of a common energetic defect able to explain the similarity of the clinical presentation. They also suggest that skin fibroblasts are interesting models to explore the metabolic defects involved in these neurodegenerative diseases. Ongoing transcriptional profiling will provide a larger overview of the metabolic perturbations associated with the diseases.

■ 3413

Mitochondrial function in LHON and OPA1 studied in vivo using MR Spectroscopy

LODI R
Dept of Clinical Medicine and Radiology, Università di Bologna, Bologna

Purpose Phosphorus MR spectroscopy (31P-MRS) has been extensively used to assess in vivo tissue mitochondrial ATP production in patients with oxidative phosphorylation deficit either with or without clinical involvement (1). In LHON patients carrying a primary mtDNA point mutation at one of three nucleotide positions, 11778, 14484 or 3460, in genes coding for different subunits of complex I of the respiratory chain we detected slow PCr recovery rate and low Vmax for mitochondrial ATP synthesis (1-3). In vivo mitochondrial activity was lowest in subjects homoplasmic for 11778, higher for 14484 and just within the normal range for 3460. Reduced brain phosphorylation potential was found in patients and asymptomatic carriers of the 11778 and 3460 mutations (2, 3). The association of "secondary" mitochondrial DNA point mutations (4216/ND1 and 13708/ND5) with the 11778/ND4 mutation does not affect the extent of brain and muscle energy metabolism deficit (2). A deficit of skeletal muscle bioenergetics similar to that found in LHON patients has been detected also in patients with autosomal dominant optic atrophy (DOA) due to the c.2708-2711delTTAG deletion in exon 27 of the OPA1 gene (4). More recently, we have shown that different mutations in the OPA1 gene consistently result in a deficit of muscle mitochondrial ATP synthesis not detected in non-OPA1 DOA patients (5). A mitochondrial dysfunction in LHON and OPA1-related DOA may be a common predisposition of neuronal cells to apoptotic death. This and other possible common consequences of mitochondrial dysfunction in LHON and OPA1-related DOA, such as reactive oxygen species overproduction or the organization of mitochondrial network in the energy-dependent unmyelinated portion of the optic nerve, need to be carefully explored to unwrap the pathogenetic peculiarities of these mitochondrial optic neuropathies.

■ 3414

Neuroprotection in inherited optic neuropathy

HOLLINS AJ (1), DAVIES VJ (1), PIECHOTA MJ (1), VOTRUBA M (1, 2)
(1) School of Optometry & Vision Sciences, Cardiff
(2) Cardiff Eye Unit, University Hospital Wales, Cardiff

Purpose Our aim is to explore the effectiveness of neuroprotective agents on opa1 mutant retinal ganglion cell (RGC) survival in vitro. Dominant optic atrophy (ADOA), mapping to the OPA1 locus, is due to heterozygous mutations in the OPA1 gene. Patients with ADOA are born with near-normal vision, but develop blindness, with onset in the first two decades. They have optic nerve pallor, centro-caecal field defects and colour deficits. Histopathological studies suggest primary RGC degeneration.

Methods Molecular, cellular and morphological characterisation of the effect of selected neuroprotective agents was optimised utilising two rat RGC cell lines (R28 and RGC5), and investigated in wild type and opa1 mutant mouse RGCs obtained by immunopanning from a novel murine model of ADOA carrying a nonsense mutation in the opa1 gene. Cells were cultured on laminin coated plates for up to 14 days, and immunofluorescently stained. MTT assay was used to assess survival under a number of conditions.

Results Immunofluorescent staining of all cell types for Thy1, Tuj 1/ Beta- III-tubulin, Brn-3b, NF-L, M and H, Tau, MAP-2, OPA1 and rabbit anti-glial GFAP confirmed the RGC identity. Opa1 mutant RGC behaviour in response to stress and a number of neuroprotective agents is described.

Conclusion Protection of RGCs is being investigated intensively in glaucoma, using a range of trophic factors, such as BDNF, CTNF, GDNF, neurotrophins 3 and 4, FGF2, NGF, as well as other substances (such as caspase 1 and 3 inhibitors and NMDA antagonists). The development of an in vitro system for studying survival of opa1 mutant RGCs will open the way towards rescue and therapy for this blinding disease.

■ 3415

The eye muscles and mitochondrial disorders

PLANT G

Moorfields Eye Hospital, London

Purpose To review the oculomotor manifestations of mitochondrial disorders.

Methods Literature review and illustrative clinical examples.

Results The extraocular muscles and the levator palpebrae are commonly involved in a variety of mitochondrial disorders. The best known is the syndrome of Chronic Progressive External Ophthalmoplegia which usually results from a mitochondrial DNA deletion and is sporadic. Kearns Sayre syndrome (KSS) has similar features but with other organ involvement (hearing, cardiac, retina, cerebellar, endocrine) more prominent. Identical mutations have been shown to cause KSS and Pearson syndrome. Autosomal dominant cases may be due to nuclear driven DNA deletions for example due to mutations of the POLG, ANT1 and C10ORF2 genes. The minimal sign of an ocular myopathy is slowing of saccades, including lid saccades. Diplopia and ptosis may require active management. Clinical features which may distinguish the disorder from myasthenia will be discussed.

Conclusion Ophthalmoplegia is a core clinical feature of a variety of mitochondrial cytopathies. Observation of the typical clinical features can be helpful in making the initial diagnosis.

■ 3421

Effects of high dose prednisolone on optic nerve head blood flow in patients with acute optic neuritis

WEIGERT G (1, 2), RESCH H (1), GARHOFER G (1, 2), KIRCHER K (2), DORNER GT (2), REITNER A (2), SCHMETTERER L (1, 3)

(1) *Clinical Pharmacology, Vienna*

(2) *Ophthalmology, Vienna*

(3) *Biomedical Engineering and Physics, Vienna*

Purpose In the present study patients with optic neuritis were treated with high dose prednisolone. Little information is available about the effects of this treatment on ocular blood flow. We set out to investigate the effects of high dose prednisolone on optic nerve head blood flow in patients with acute optic neuritis.

Methods Nine patients with acute optic neuritis were included in the study. 1 g of prednisolone was infused intravenously over 30 minutes on three consecutive days. On each study day optic nerve head blood flow (ONHBF) was measured using laser Doppler flowmetry. The ocular hemodynamic measurements were performed on the contralateral eye of the patients with optic neuritis before and immediately after cessation of the infusion. Intraocular pressure and systemic blood pressure was measured before and after the infusion on each study day. Data was analyzed using a 3x2 repeated measures ANOVA model.

Results Prednisolone increased optic nerve head blood flow (ONHBF) in the patients under study ($p < 0.05$). No significant change in mean arterial pressure (MAP) ($p = 0.14$) or intraocular pressure (IOP) ($p = 0.91$) could be detected in the patients treated with high dose prednisolone.

Conclusion High dose prednisolone showed a small but significant increase in optic nerve head blood flow. Further studies are required to study whether this effect contributes to the therapeutic efficacy of cortisone in patients with optic neuritis.

■ 3422

Involvement of the dopamine-1 receptor in choroidal blood flow changes during light/dark transitions

FUCHSJÄGER-MAYRL G (1, 2), HUEMER K-H (3, 1), KARL K (1), RESCH H (1), WEIGERT G (1), POLSKA E (1), GARHOFER G (1), SCHMETTERER L (1, 4)

(1) *Clinical Pharmacology, Vienna*

(2) *Ophthalmology, Vienna*

(3) *Physiology, Vienna*

(4) *Biomedical Engineering and Medical Physics, Vienna*

Purpose Recent results indicate that a light/dark transition is associated with a reduction in choroidal blood flow (CBF) due to an unknown mechanism. Dopamine has been discussed as a chemical messenger for light adaptation. Accordingly, dopamine could provide a modulatory input to the light/dark transition induced changes of choroidal circulation.

Methods 12 healthy male subjects were included in this randomized placebo-controlled three way cross-over trial. On three different study days the D1 antagonist quetiapine, the D2 antagonist sulpiride or placebo were administered. CBF was measured using laser Doppler flowmetry during light/dark transitions and the results were expressed as %change from baseline. This dark reactivity was compared among the three study days using a one-way ANOVA.

Results In keeping with our previous studies CBF showed a reversible decrease after light/dark transition. None of the administered drugs changed baseline CBF. The dark reactivity was $11.5 \pm 5.8\%$ on the placebo study day. This dark reactivity was significantly altered after administration of quetiapine ($5.8 \pm 4.6\%$, $p < 0.05$), whereas sulpiride did not alter the dark reactivity ($9.4 \pm 7.1\%$).

Conclusion We have previously shown that dopamine increases CBF in healthy subjects. The results of the present study indicate that neither D1- nor D2-receptor antagonists alter CBF at baseline. The D1 receptor antagonist quetiapine does, however, alter the response of CBF during light/dark transitions indicating a role of this receptor subtype in the regulation of blood flow during changes in retinal illumination.

■ 3423

Effects of yohimbine on dynamic autoregulation.

POLSKA E (1), KOLODJASCHNA J (1), BERISHA F (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 In the present study we investigated if the response of blood flow velocities in the ophthalmic and middle cerebral artery (OA, MCA) to the drop in blood pressure is altered by administration of the alpha-2 antagonist yohimbine.

Methods 16 healthy nonsmoking male subjects were studied in a randomized placebo controlled design on 2 study days. Ultrasound parameters and arterial blood pressure (ABP) were recorded before, during, and after a step drop in blood pressure after administration of either yohimbine or placebo. A thigh cuff technique was used to induce the drop in ABP. Peak systolic and end diastolic flow velocity (PSV, EDV) were assessed with a color Doppler probe. Systolic and diastolic blood pressures (SBP, DBP) were measured continuously using a Finapres system.

Results The time course of blood flow velocity in response to the thigh cuff release was statistically significant for systole and for diastole versus baseline in the both arteries. In addition, we found a statistically difference in the response of the time course of flow velocities and autoregulatory indices between the OA and the MCA. Autoregulatory indices indicate an early vasodilatation distal to the MCA and a slightly retarded vasoconstriction distal to the OA. The latter effect was reduced by administration of yohimbine ($p = 0.03$), whereas the response in the MCA was not modified by the alpha-2 antagonist.

Conclusion Our results confirm differences between the autoregulatory response of the MCA and the OA after a step decrease in perfusion pressure. Modification of the dynamic response of flow velocities in the OA by yohimbine indicate that vasoconstriction after a step decrease in blood pressure is related to a change in sympathetic drive mediated at least in part via alpha-2 receptors.

■ 3424

Nitric Oxide and Choroidal Blood Flow in Healthy Subjects

GARHOFER G (1, 2), BERISHA F (1), FUCHSJÄGER-MAYRL G (1, 2), WOLZTM (1), SCHMETTERER L (1, 3)

(1) *Department of Clinical Pharmacology, Vienna*

(2) *Department of Ophthalmology, Vienna*

(3) *Department of Biomedical Engineering and Medical Physics, Vienna*

Purpose In the recent years we have performed a number of studies investigating the effects of systemic nitric oxide synthase (NOS) inhibition with L-NMMA on choroidal blood flow regulation in healthy volunteers. The present analysis was done to investigate whether the response in fundus pulsation amplitude (FPA) and mean arterial pressure (MAP) to L-NMMA was dependent on the baseline values.

Methods 67 healthy male subjects were included in this analysis. All subjects received systemic L-NMMA in a dose of 3 mg/kg as a bolus infusion. These volunteers participated in a number of clinical trials that were designed to elucidate the role of nitric oxide in choroidal blood flow regulation. In all subjects FPA and MAP was measured immediately before and 5 minutes after bolus administration. A linear regression analysis was performed to calculate a potential association between the baseline FPA and the % change in FPA after L-NMMA administration and baseline MAP and the % change in MAP after L-NMMA administration.

Results As expected L-NMMA reduced FPA and increased MAP. In addition, a negative correlation was found between baseline FPA and the L-NMMA-induced change in FPA ($P = 0.007$) as well as between baseline MAP and the L-NMMA induced change in MAP ($P < 0.001$).

Conclusion The reduction in FPA and the increase in MAP after administration of L-NMMA indicates that nitric oxide maintains vascular tone in the ocular and systemic vascular circulation. In addition, the negative association between the response to L-NMMA and the baseline data indicates that even under physiological conditions higher NO production may be associated with lower blood pressure and higher choroidal blood flow.

■ 3425 / 445

Choroidal blood flow of patients undergoing an hemodilution for retinal vein occlusion

CHIQUET C (1), ROMANET JP (1), SAVY O (1), KUCHLER F (2), DRILLAT P (3), VINH V (1), TONINI M (1), GEISER MH (4, 2)

(1) Department of Ophthalmology, Grenoble

(2) Institut de Recherche en Ophtalmologie, Sion

(3) Etablissement Français du Sang, Grenoble

(4) Haute Ecole Valaisanne, Sion

Purpose To evaluate the effect of hemodilution on sub-foveal choroidal blood flow (ChBF) in the human eye with retinal vein occlusion

Methods ChBF was measured by laser Doppler flowmetry (LDF) in 16 patients (54 ±13 years, SD, between 22 and 81 years old) with retinal vein occlusion (RVO) in one eye. Isovolemic hemodilution was performed in patients with RVO after informed consent, when hematocrit was higher than 35%. ChBF was measured on both eyes 1 day before hemodilution, 1 one hour before each hemodilution and 1 hour later. Criterion to validate the measurement is the reproducibility of the amount of backscattered light from the fundus which is about three order of magnitude higher than the Doppler signal. LDF parameters were the flow (ChBF), the velocity (ChBVel) and the volume (ChBVol).

Results After hemodilution, hematocrit was reduced by 22% ($p<0.0001$). In healthy eyes ($n = 22$), hemodilution leads to an increase of ChBVel (7%) and ChBF (7%). In eyes with RVO ($n = 16$), the reduction of hematocrit was associated with a reduction of ChBVel (5%) and ChBF (6%). In both eyes, ChBVol did not change significantly. Comparison of the changes for each patient between affected eyes and contralateral healthy eyes was significant for ChBVel and ChBF.

Conclusion After isovolemic hemodilution, a significant decrease of hematocrit induced an opposite change of ChBF and ChBVel between affected eyes and contralateral healthy eyes. These preliminary results must be confirmed with a larger series of patients. Furthermore changes of choroidal blood flow parameters may not reflect changes in the retinal circulation.

■ 3426 / 446

The effects of intravenous histamine and histamine-receptor blockade on ocular blood flow

KARL K (1), RESCH H (1), WEIGERT G (1, 2), ZAWINKA C (3), GARHOFER G (1, 2), SCHMETTERER L (1, 4)

(1) Clinical Pharmacology, Vienna

(2) Ophthalmology, Vienna

(3) University Eye Clinic, Basel

(4) Biomedical Engineering and Medical Physics, Vienna

Purpose To investigate the effect of intravenous administered histamine on ocular blood flow and to identify the receptors mediating the histamine induced blood flow response.

Methods In study A placebo or histamine was administrated intravenously. In studies B and C histamine was infused in the absence or presence of the H1-receptor-antagonist diphenhydramine or the H2-receptor-antagonist cimetidine, respectively. In all three studies, choroidal blood flow was assessed with laser Doppler flowmetry. Retinal blood flow was calculated based on measurements of retinal vessel diameters using a retinal vessel analyzer and retinal blood cell velocity assessed with laser Doppler velocimetry.

Results Study A: Histamine significantly increased retinal arterial and venous diameters, whereas retinal blood flow was not affected. Choroidal blood flow increased after administration of histamine. Studies B and C: Co-administration of cimetidine did not modify the effects of histamine on ocular blood flow. In contrast, co-administration of diphenhydramine significantly reduced histamine-induced blood flow effects.

Conclusion Histamine induces an increase in choroidal blood flow as well as a vasodilatation in retinal arteries and veins. This effect can be blunted by co-administration of a H1 receptor antagonist. Our results strongly suggest that in the human retina and choroid histamine induced vasodilator effects are mediated by H1-receptors.

■ 3427 / 447

Effects of Indomethacin on Retinal and Choroidal Blood Flow in Healthy Volunteers

BERISHA F (1, 2, 3), WEIGERT G (1, 4), FUCHSJÄGER-MAYRL G (1, 4)

SCHMETTERER L (1, 5), GARHOFER G (1, 4)

(1) Department of Clinical Pharmacology, Vienna

(2) Schepens Retina Associates Foundation, Boston

(3) Ophthalmology, Harvard Medical School, Boston

(4) Department of Ophthalmology, Vienna

(5) Center for Biomedical Engineering & Physics, Vienna

Purpose Prostaglandins are assumed to play a role in ocular blood flow regulation. Animal studies suggest that retinal and choroidal blood flow decrease after administration of indomethacin, a nonspecific cyclooxygenase inhibitor. The aim of this study was to investigate the effects of indomethacin on ocular blood flow in healthy humans.

Methods Ten healthy male volunteers were studied in a randomized double-masked, placebo-controlled, two-way crossover design. Indomethacin or placebo was administered intravenously as a bolus (0.4 mg/kg) followed by continuous infusion of 0.4 mg/kg/h over 2 hours in two different study days. Ocular hemodynamics were measured at baseline, 30 minutes, 1, 2 and 3 hours after the start of the infusion. Retinal vessel diameters were assessed using a retinal vessel analyzer; retinal blood velocity was measured using the bidirectional laser Doppler velocimeter. Retinal blood flow was calculated based on retinal vessel diameter and red blood cell speed. Subfoveal choroidal blood flow was measured using laser Doppler flowmetry.

Results Indomethacin significantly decreased retinal blood velocity by -16±37% ($p=0.0359$ versus placebo) and retinal blood flow by -29±24% ($p<0.01$ versus placebo). Choroidal blood flow was also significantly decreased by -16±16% after indomethacin administration ($p=0.03$ versus placebo).

Conclusion Our results showed a marked decrease in retinal and choroidal blood flow, indicating that prostaglandins are involved in the regulation of ocular blood flow. The mechanisms underlying the decreasing effects of indomethacin on ocular blood flow remain to be clarified.

■ 3431

Ocular sebaceous carcinomas: differential diagnosis in clinical and pathological presentation

DE KEIZER RJW (1, 2), DE WOLFF ROJENDAAL D (1)

(1) Ophthalmology LUMC ocular oncology unit, Leiden

(2) Ophthalmology, Antwerp

Purpose Sebaceous gland carcinoma in the western world is a rare, potentially fatal disease, increasing in incidence. It is very important to differentiate it from other eyelid lesions.

Methods In a retrospective study we analysed our sebaceous gland carcinoma data of our ocular oncology unit with special attention to the clinical presentation and histopathological findings.

Results From the total 12 cases, 9 revealed a sebaceous carcinoma in a Meibomian gland, 3 in a Zeis gland and one in the caruncula. Two presented as a cyst, 2 were multicentric, 3 consisted of a nodule and pagetoid extension in the conjunctiva. Six tumours masqueraded as CIN, BCC and SCC or one-sided chronic blepharoconjunctivitis. In four tumours the original histological diagnosis had to be revised. Ten cases were treated by local excision. In one case a local excision was done followed by an eyelid reconstruction but after the presentation of a second sebaceous gland carcinoma orbital exenteration was performed. In one case a local excision was done in conjunction with Mitomycin-C courses and local radiotherapy. The follow-up was 1-10 years, without tumour deaths.

Conclusion Based on our clinical and pathological results, we conclude that sebaceous gland carcinomas vary considerably in clinical presentation and histopathological findings. The final diagnosis could only be made after communication between ophthalmic or dermatological pathologist and ocular oncologist.

■ 3432

Sebaceous carcinoma in a spanish series of eyelid tumors

MENDEZ MC (1), SARASA JL (2), SAORNIL MA (1), BLANCO G (1)

(1) Registro de Patología Ocular Miguel N. Burnier. Instituto Universitario de Oftalmobiología Aplicada (IOBA), Valladolid

(2) Departamento de Anatomía Patológica. Fundación Jiménez Díaz, Madrid

Purpose To present four cases of sebaceous carcinomas of the eyelid, and emphasize the low incidence of this type of carcinoma in Spain compared to previous published series.

Methods Four cases of eyelid sebaceous carcinomas have been observed in two Spanish institutions during the last 14 years, among more than 1500 eyelid tumors. Clinic and pathologic characteristics are analysed.

Results The patients were one male and three females, older than 50 years (mean age of 73 years, ranging from 59 to 96 years). Clinical presentation was masquerade as an inflammatory lesion mimicking a chalazion in one case, as a cutaneous horn in the second case and in the other two cases as eyelid nodules, clinically misdiagnosed as basal cell carcinomas. Location was one case in the upper and one in the lower eyelid, third case in the external cantus and the remaining tumor in the eyelid margin. The tumor placed in the external cantus was large, with a diameter of 16 mm, being the other cases much more smaller. Sensitivity of clinical diagnosis for these tumors was low: none of the four cases was clinically diagnosed as sebaceous carcinoma.

Conclusion Sebaceous carcinoma of the eyelid has been described in the literature as the third malignant tumor in frequency after basal cell and squamous cell carcinomas. The incidence of the sebaceous cell carcinoma in our country is smaller than the described in the literature, being the basal cell carcinoma, the squamous cell carcinoma and the malignant melanoma the most frequent tumors (in this order) in our series of eyelid tumors.*Partially funded by grant "Jose María Aguilar Bartolomé"

■ 3433

Caruncular lesions in Denmark 1978-2002. A histopathological study with correlation to clinical referral diagnosis

HEEGAARD S (1), ØSTERGAARD J (1), PRAUSE JU (1, 2)

(1) Eye Pathology Institute, Copenhagen

(2) Department of Ophthalmology, Rigshospitalet, Copenhagen

Purpose To carry out a retrospective clinicopathological evaluation of caruncular lesions.

Methods Data were collected from all surgically removed and histopathologically evaluated caruncular lesions registered by Danish pathology departments during the 25-year-period 1978 to 2002.

Results A total of 574 caruncular lesions were identified. The number of caruncular lesions increased significantly during the 25-year-period. This was due to an increase in number of benign lesions, whereas the number of premalignant and malignant lesions remained constant. A total of 550 (96 %) of the lesions were benign. Naevus (248; 43 %) and papilloma (131; 23 %) were the most common neoplasms. Premalignant lesions (10; 1.7 %) were dominated by primary acquired melanosis (PAM) with atypia and epithelial dysplasia. Malignant lesions constituted a total of 14 neoplasms (2.4 %), with basal cell carcinoma (4; 0.7 %) and lymphoma (4; 0.7 %) being the most frequent. The preoperative clinical diagnosis was correct in 286 (50 %) of the cases.

Conclusion Caruncular lesions are predominately benign, but the lesions are rare and diverse, making clinical diagnosis difficult. Referral of excised lesions for pathological examination is recommended.

■ 3434

Screening for Optic Pathway Gliomas in Children with Type 1 Neurofibromatosis

VELUSAMI P (1), SANDHUS (2), CLARKE M (2)

(1) Queen Mary's Hospital, Sidcup

(2) Royal Victoria Infirmary, Newcastle

Purpose Review of the experience of screening for Optic Pathway Gliomas (OPG) in children with type 1 neurofibromatosis (NF1).

Methods The North of England's Young Persons Malignant Disease Registry was searched from 1975 to 2005 for records of children in North England diagnosed with OPG and NF1.

Results Over a 30 year period, 22 patients with OPG and NF1 were identified. Median age at diagnosis = 5.9 years (range 2 – 18.5), follow up 3 – 16 years. 64% were symptomatic at presentation, 36% were asymptomatic. Symptomatic children reported decreased vision (47%), headache (33%), squint (20%), proptosis (13%) and photophobia (7%). Mean duration of symptoms = 6.3 months (range 0.25 – 12 months). Asymptomatic children were identified by disc pallor (50%), visual field defects (25%) and decreased colour vision (25%). 6 cases were incidental findings on routine examination by paediatrics, whilst 2 cases were identified by ophthalmic screening. Location of the OPG was intraorbital (18%), chiasmal only (23%), optic nerve and chiasmal (32%), chiasmal and optic tract (9%) and bilateral (18%). Treatment included conservative management (68%), radiotherapy (23%), chemotherapy (5%) and operative (9%). Only children with chiasmal involvement and deteriorating vision underwent intervention. All asymptomatic children were managed conservatively.

Conclusion Only clinically symptomatic OPG underwent active management. All asymptomatic children received no active intervention. The value of annual screening of children with NF1 for OPG by clinical ophthalmic examination is debatable. An alternative strategy might be ophthalmic examination on diagnosis with improved parental information on ocular complications of NF1 and subsequent presentation if symptoms arise.

■ 3435

A young man with progressive visual loss and an optic disc tumour

ALJAMAL R, KIVELÄ T

Ophthalmology and Ocular Oncology, Helsinki University Central Hospital, Helsinki

Purpose This case is presented for diagnosis and discussion.

Methods A 24-year-old healthy army officer presented with a one-week history of painless decrease of vision to 20/60 in his right eye. Three months earlier, he had had bilateral conjunctivitis, otherwise his history was noncontributory. His pupil was normally reactive and the anterior segment was normal, but posterior segment examination revealed a swollen optic disc, venous congestion and small hemorrhages around it. Vitreous clouding was noticed in the inferior part of the fundus. Visual field examination re-vealed a central scotoma. Color vision testing showed a few errors. Optic neuritis was suspected. An MRI was done to rule out multiple sclerosis but was normal. ESR, WBC, HIV, borrelia and CMV anti-bodies as well as other blood tests were normal, except for a borderline ANA test.

Results Three weeks later vision was 20/50 and venous congestion was milder. He was scheduled for monthly follow up, but turned up only three months later. Vision of the left eye was now counting finger at 3 meters, and a large tumor was found in front of the optic disc, measuring 8.1 mm in diameter and 3.8 mm in thickness, surrounded by an exudative retinal detachment and dot and blot hemorrhages. A repeat MRI showed a normal orbit and brain except for a mass in the area of the optic nerve head. The patient was referred to an ocular oncologist for a suspected optic nerve malignancy.

Conclusion The case is presented for diagnosis and discussion.

■ 3436

Progesterone receptor and c-kit expression in orbital cavernous hemangiomas

BRISCOE D (1), ROSEN E (1), NESHER R (1), KIDRON D (2)

(1) Ophthalmology, Meir Medical Center, Kfar Saba

(2) Pathology, Meir Medical Center, Kfar Saba

Purpose Cavernous hemangioma is the most common benign adult tumor of the orbit. Hormonal changes can effect vascular neoplasms, and in one single case series reported in the literature progesterone receptors were found present in all orbital cavernous hemangiomas. The C kit protein is a tyrosine kinase growth factor that stimulates cancerous tissue cell growth, and has been associated specifically with gastrointestinal stromal tumors among other neoplasms. The purpose of our study was to examine the presence of Progesterone receptors and the C - kit protein in 14 cases of orbital cavernous hemangioma.

Methods 14 cases operated in Meir Medical Center between 1999 and 2005 were included in the study. The patients, age, sex and tumour size were recorded and all cavernous hemangiomas underwent automated immunohistochemical staining for progesterone receptors and C- kit protein.

Results There were 7 males and 7 females, with age ranging between 31 and 70 years old. Size of tumours after fixation in formaline ranged between 0.4 X 1.0 X 1.5 and 1.8X1.8X 3.5 cm. Staining for progesterone receptors was strongly positive in 6 cases, weakly positive in 6 cases and negative in 2 cases. C- kit staining was positive in all 14 cases.

Conclusion We found that the presence of progesterone receptors varies in orbital cavernous hemangiomas and our results therefore differ from those published previously. To the best of our knowledge the presence of C-kit antibodies in orbital cavernous hemangiomas has not previously been published, and our study demonstrates their presence in all of our cases. These findings may have future clinical and treatment implications in the management of these tumors.

■ 3437 / 477

Merkel cell carcinoma of the eyelid with two cases of spontaneous regressionMISSOTTEN GS, DE WOLFF-ROUENDAAL D, DE KEIZER RJW
Ophthalmology, Leiden University, Leiden

Purpose To evaluate the clinical and histopathological characteristics of Merkel cell carcinoma (MCC) of the eyelid and to report cases of spontaneous regression of a MCC after biopsy. The Merkel cells are present in the epidermis and are associated with touch receptors.

Methods Clinical records and histopathological material of patients with eyelid Merkel cell carcinoma were reviewed. The clinical presentation and treatment were evaluated.

Results Three cases of Merkel cell carcinoma of the eyelid were found. Diagnosis was made by pathologic investigation and immunohistochemistry (S100, CK20, EMA, chromogranin). Clinical differential diagnosis must be made with a chalazion, and histopathological differential diagnosis with small cell carcinomas. One of the cases showed complete spontaneous regression after biopsy of the tumour. After local excision, none of the MCC had local recurrence, nor regional or distant metastases. Mean clinical follow-up was 50 months. Non-ocular MCC is known to recur in 66% of cases and to give mortality in almost 33%.

Conclusion Merkel cell carcinoma of the eyelid is a rare malignancy, which is not recognised clinically. It should be excised with a large margin (> 3cm). Due to its high rate of recurrence and lymphatic spread, a tight surveillance of these patients remains necessary. The immunological phenomenon of spontaneous regression points out the importance of the immune system in this disease.

■ 3438 / 478

Vascular features of mushroom-shaped uveal melanomas

AMIRYAN AG (1), SAAKYAN SV (1), LELIUK VG (2)

(1) Ocular Oncology and Radiology, Moscow Helmholtz Research Institute of Eye Diseases, Moscow

(2) Clinical Physiology Laboratory, State Research Center – Institute of Biophysics, Moscow

Purpose To study the vascular features of mushroom-shaped uveal melanomas.

Methods 15 patients with mushroom-shaped uveal melanomas (8 men and 7 women; age varied from 19 to 72 years) were examined using high-frequency (10-13 MHz) duplex ultrasonography with Color Doppler imaging (CDI) and spectral Doppler analysis. The examination was performed using Sequoia-512 (Siemens AG, Germany) with linear array 15L8w. Tumor thickness average was 7.7 ± 2.6 mm (4.3 - 12.4).

Results Studying mushroom-shaped uveal melanomas by CDI, we determined two types of tumor's vascularization. In the eyes with the first type of mushroom-shaped melanomas (8 persons) vascular patterns were detected at the base and in the neck of tumors, whereas there was no abnormal blood flow signals in the head marked. At the patients with the second type of vascularization (7 persons) uniform distribution of Doppler signals in the tumor were visualized. The first type of mushroom-shaped uveal melanomas characterized by significantly more higher peak systolic velocities and lower peripheral resistance in the tumor vessels than the second one.

Conclusion The received Doppler characteristics distinctions of blood flow in the submitted vascular types of mushroom-shaped uveal melanomas could testify the greater tumor activity at the patients with the first type of vascularization. This technique may be valuable as an additional noninvasive tool for the selection of therapy methods the patients with mushroom-shaped uveal melanomas.

■ 3441

Attention effects in visual texture segregation

HEINRICH SP (1), ANDRES M (2), BACH M (1)

(1) Univ.-Augenklinik Freiburg, Sektion Funktionelle Sehforschung, Freiburg
(2) Albert-Ludwigs-Universität, Fakultät für Biologie, Freiburg

Purpose Visual texture segregation is believed to be performed pre-attentively. Recent evidence, however, challenges this view. Using visual evoked potentials (VEPs), we investigated the effect of different tasks on texture segregation.

Methods Stimuli consisted of Gabor-filtered binary noise patterns. In segregated stimuli, local texture orientation contrasts defined global checkerboard patterns. VEP responses specific to texture segregation were obtained by computing the difference between VEPs to homogeneous and segregated stimuli. Four conditions were examined that required attending either the global pattern, the local structure, random numbers displayed on the screen, or a series of tones.

Results Responses specific to texture segregation were dominated by two occipital negativities peaking around 110 ms and 230 ms. The earlier one was not affected by the task, while the later one was completely abolished when the subjects attended to either numbers or tones ($p=0.005$ and $p=0.006$, respectively).

Conclusion The results suggest that early stages of texture segregation are not affected by attention, while task relevance is crucial for later processes. The timing is compatible to a recurrent processing pattern with initial bottom-up processing of basic stimulus characteristics and a subsequent top-down flow of higher-level modulatory information.

■ 3442

Local motion discrimination deficits in ocular hypertension

FARIA P (1, 2), SILVA MF (2), CASTELO-BRANCO M (2)

(1) Responsibility Center of Ophthalmology – University Hospital of Coimbra, Coimbra
(2) Institute of Biomedical Research of Light and Image – Faculty of Medicine – University of Coimbra, Coimbra

Purpose Both local and global motion perception deficits have been described in glaucoma. The goal of this work was to evaluate local speed discrimination deficits in individuals with ocular hypertension using a psychophysical technique that provides relative isolation of the magnocellular pathway.

Methods A computerized Local Speed Discrimination Task, based on velocity comparisons of two widely separated dots, moving with random trajectories, was used to measure local motion sensitivity across four meridians (horizontal, vertical, 45 and 135°) with eccentricities between 7.5 and 15°. Study population – Individuals with ocular hypertension (n=16 eyes), with exclusion of any other ocular pathology. Control group: individuals with normal ophthalmological examination (n=16 eyes).

Results Local speed discrimination thresholds were significantly higher in the ocular hypertensive group, comparing the control group, for the following meridians: 45° ($p=0.0169$, Mann-Whitney Test), 135° ($p=0.0179$) and vertical ($p=0.044$). At the horizontal meridian, and a relatively small (7.5°) eccentricity, the effect did not reach statistical significance. ($p=0.3047$).

Conclusion Our results indicate the presence of local speed discrimination deficits in ocular hypertension, in outer macular regions (up to 15°) regions of the visual field. This suggests that local speed discrimination deficits are a good indicator of early involvement of the visual magnocellular pathway in glaucoma.

■ 3443

Assessment of chromatic sensitivity loss in subjects with unusual congenital deficiencies - a study of the factors that affect anomaloscope matches

BARBUR JL, RODRIGUEZ-CARMONA ML, HARLOW JA

Applied Vision Research Centre, London

Purpose The two most sensitive techniques for assessing congenital colour deficiency and / or acquired changes in chromatic sensitivity involve either measurement of chromatic discrimination thresholds or anomaloscope matches. These techniques also make it possible to quantify the severity of colour vision loss, but the large variability within normal trichromats and the existence of unusual, extreme anomalous matches limit their usefulness. The purpose of this investigation was to examine the factors that cause changes in chromatic sensitivity in order to explain the inherent variability within normal trichromats and the existence of unusual extreme anomaloscope matches.

Methods We measured and compared colour discrimination thresholds and Nagel anomaloscope matches in a large number of "normal" trichromats and a group of colour deficient observers. Genetic analysis was also carried out in a subgroup of subjects to determine the spectral separation between the L and M pigments. We also modelled the Nagel match by considering how changes in wavelength separation, post-receptoral amplification of cone signals and optical density changes affect the parameters of the "yellow" match.

Results The results define the "normal" statistical limits of chromatic sensitivity and explain and predict the existence of extreme anomaloscope matches.

Conclusion The parameters of the yellow match can be predicted accurately both within normal trichromats and in extreme anomalous subjects by a suitable choice of wavelength separation, post-receptoral amplification and cone optical densities.

■ 3444

Subretinal Chronic Multi-Electrode Arrays Implanted in Blind Patients

ZRENNER E (1), BESCH D (1), BARTZ-SCHMIDT KU (1), GEKELER F (1),

GABEL VP (2), KUTTENKEULER C (1), SACHS H (2), SAILER H (3),

WILHELM B (4), WILKER R (1)

(1) University Eye Hospital, Tuebingen

(2) University Eye Clinic, Regensburg

(3) Retina Implant GmbH, Reutlingen

(4) STC Autonomous Nervous System and Safety Studies, Offenbourg

Purpose Assessment of function of subretinal implants consisting of a chip (3x3x0.1mm, 1500 microphotodiodes, amplifiers and electrodes of 50x50 µm, spaced 70 µm) and a 4x4 array of identical electrodes spaced 280 µm for direct stimulation (DS), chronically implanted next to the foveal rim of 2 blind RP patients.

Methods Chip and DS array are positioned on a subretinal polyimide foil powered via a transchoroidal, retroauricular transdermal line ending in a radio-controlled, battery-driven receiver box.

Results The implants were well tolerated without adverse events. OCTs showed stable attachment of the retina. As per study plan, the implant was removed in one patient after 4 weeks. The other patient chose to keep the implant. Charge injection delivered by 16 DS electrodes was simultaneous or successive to present temporal or spatial patterns. Patients reported small, yellowish or greyish phosphenes for individual electrode stimulation. They were able to differentiate spatial patterns such as lines, angles or bright squares. Simultaneous stimulation of four electrodes in a line or a row was reported as "bright yellowish rod with round corners, the size of a match." The patient was able to clearly distinguish horizontal from vertical lines and to correctly describe the alignment and direction of dot movement if three or four neighbouring electrodes were switched on sequentially at one-second intervals.

Conclusion Chronic subretinal stimulation via small electrodes spaced approx. 1° allows the discrimination of patterns of dots, reliably repeated over weeks via a well-tolerated, still intact subretinal microelectrode array. Supported by BMBF 01KP0008 and Retina Implant GmbH

■ 3445

Differences in ametropia prevalence obtained different methods of refraction

JORGE J (1), GONZALEZ-MEIJOME JM (1), QUEIROS A (1),
BORGES DE ALMEIDA J (2), PARAFITA MA (3)

(1) *Physics Optometry, Braga*

(2) *Physics, Braga*

(3) *Surgery Ophthalmology, Santiago de Compostela*

Purpose This study was carried out to establish the ideal cut-off point (COP) for cycloplegic (C) and non-cycloplegic (NC) refraction with different methods against subjective cycloplegic refraction (Sx) considered as the "gold standard"

Methods A population sample of 199 young adults were randomly selected and examined with autorefraction (AR), retinoscopy (Rx) and subjective refraction (Sx). The results obtained were converted into spherical equivalent refraction (SER) to classify myopia (M), emmetropia and hyperopia (H)

Results The prevalence of M and H obtained with CSx was 20.6% and 49.7%, respectively. For all methods of measurements using cycloplegia there were not statistically significant differences in the prevalence of ametropia. For the CAR the prevalence was 21.6% and 50.8% for M and H respectively ($p=0.808$) and 20.6% and 45.2% for M and H respectively ($p=0.343$) obtained with CRx. For the NCSx, NCRx and NCAR the COP of ± 0.50 D do not reflect the actual prevalence of the ametropia as obtained with CSx. For the NCAR the ideal COP for M was SER ≤ 1.25 D and SER ≥ 0.25 for H ($p=0.148$). For the NCSx and NCRx the ideal COP for M and H were SER < 0.50 D ($p=0.057$) and SER ≥ 0.25 ($p=0.052$), respectively

Conclusion For a population of Caucasian young adults, similar prevalence of refractive conditions were found under cycloplegic conditions irrespective of the method used. However, under non-cycloplegic conditions, it is necessary to change cut-off points by at least 0.50 D in order to meet similar results than those found using the gold standard. World wide uniformization of methods to define ametropia is mandatory to obtain more accurate information on the impact of myopia among different ethnic groups.

■ 3446

Accommodative changes as measured by optical biometry

LANZL I, NURISPAHIC A, KOTLIAR KE
Eye Clinic, Technical University, Munich

Purpose Accommodation for all age groups is of large interest. To study the physiologic mechanisms we applied optical biometry. Longitudinal relations in the optical axis during accommodation and desaccommodation in volunteers of different age and lens status were studied.

Methods 3 groups of 15 emmetropic subjects of 10.5 ± 1.9 , 21.9 ± 2.1 and 55.7 ± 7.3 years each and 15 pseudophakic patients of 59.9 ± 7.8 years were examined by IOL-Master. A custom made special unit stimulating accommodation either in the ipsilateral or contralateral eye during measurement was used to obtain near accommodation or distance desaccommodation. Reproducibility of the method was tested by repeat testing and by using 2 observers.

Results Results of repeat testing and of 2 different observers correlated significantly ($r > 0.98$, $p < 0.004$). In the desaccommodative state anterior chamber depth (ACD) increased with age from children (3.62 ± 0.19 mm) to young adults (3.76 ± 0.23 mm) with a significant decrease in older adults (3.21 ± 0.33 mm). Near accommodation in young adults induced the largest ACD decrease (0.14 ± 0.03 mm) followed by children (0.11 ± 0.03 mm) and older adults (0.07 ± 0.02 mm) and no change in pseudophakic patients. Axial length increased in all groups during near accommodation by 0.01 ± 0.01 mm ($p < 0.01$).

Conclusion In children and young adults pronounced changes in ACD were measured in comparison to older adults reflecting the curvature change of the lens during accommodation. The measured anterior posterior elongation of 0.01 mm in some subjects may be attributed to IOL-Master accuracy in regard to oscillations due to the cardiac cycle and breathing. At near sighted stimulus we did not find any changes in anterior chamber depth and thus lens position in pseudophakic patients compared to desaccommodation.

■ 3447

Functional visual performance assessed with and without correction of ocular aberrations

DALIMIER E (1), DAINTY JC (1), BARBUR JL (2)

(1) *Applied Optics Group, National University of Ireland, Galway*
(2) *Applied Vision Research Centre, City University, London*

Purpose Adaptive Optics (AO) technology enables us to dynamically compensate for ocular aberrations beyond spherocylindrical errors that are corrected traditionally with ophthalmic prescriptions or laser surgery. The visual benefit gained with AO has recently been demonstrated for dilated eyes under photopic conditions. We have extended these studies by investigating the effect of AO on functional visual performance under a range of light levels that cover the photopic and the mesopic range.

Methods An AO system was designed to correct dynamically the higher-order aberrations of the eye. The system also included facilities for the measurement of contrast acuity. The initial study was restricted to a group of normal, young subjects with good vision. A QUEST psychophysical procedure was used to measure contrast acuity threshold under different background light adaptation conditions with and without AO correction of higher-order aberrations.

Results The AO system produced good correction of higher-order aberrations and made it possible to measure the relation between AO visual benefit and retinal illuminance. Measurements with dilated and natural pupils were compared.

Conclusion The use of a combined AO / optical stimulator system made it possible to investigate how changes in natural pupil size, ocular aberrations and the resolution of the retina and visual pathways affect visual performance as a function of light level. The preliminary findings suggest that correction of higher order aberrations benefits visual performance for large pupil sizes under photopic conditions. The results also suggest that the resolving power of the retina is a factor that limits visual performance in the mesopic range.

■ 3451**Comparative study of two silicone hydrogel contact lenses used as bandage after LASEK**

GIL-CAZORLA R (1, 2), TORRES J (1), HERNANDEZ-VERDEJO JL (1, 2), ARRANZ E (1), TELIS MA (1, 3, 4)

(1) Visum Corporación Ofthalmológica De Madrid, Madrid

(2) Escuela Universitaria De Optica (UCM), Madrid

(3) Universidad de Niversidad DE Alcalá, Alcalá De Henares

(4) Hospital Príncipe De Asturias, Alcalá De Henares

Purpose To evaluate the clinical performance of two different silicone hydrogel contact lenses used as a continuous-wear bandage contact lens after LASEK surgery.

Methods This is a prospective, single masked, interventional study. The inclusion criteria were myopia lower ≤ -6.00 D, astigmatism lower than ≤ -1.5 D; BCVA $\geq 20/20$, in both eyes. Patients were randomized fitted with PureVision in one eye and Acuvue Advance in the other. High contrast visual acuity, corneal epithelium integrity, conjunctival hyperemia, lens movement, contact lens debris and subjective comfort questionnaire were assessed. Patients wore continuously both contact lenses for 5 days. Measures were taken before and at 1 day and 5 days after the procedure. The unpaired Student's T test analyses was performed.

Results We analyzed 44 eyes of 22 consecutive patients who underwent LASEK. There was no difference in any measurement except tearing (greater with PureVision) 24 hours after the surgery ($p = 0.01$). In contrast, contact lens debris and comfort were statistically better in Acuvue Advance contact lens group at 5 days after LASEK ($p = 0.01$ respectively).

Conclusion Our results suggest that PureVision and Acuvue Advance contact lenses are useful used as continuous wear bandage. There is no difference in epithelium integrity amongst lenses although patients report better comfort with Acuve Advance.

■ 3452**Biometric changes in the anterior eye with orthokeratology**

OWENS H, WONG E, LO JP

Optometry & Vision Science, Auckland

Purpose To compare the ocular biometric responses to one night of orthokeratology (OK) lens wear with a conventionally designed lens of equivalent oxygen permeability.

Methods Nine, young myopic subjects participated in the study. Each subject wore a reverse geometry OK lens (Ultravision, Brisbane, Australia) in one eye for one night. The contralateral eye was used as a control, wearing a conventional lens of equivalent dimensions and oxygen permeability. Anterior corneal radius of curvature, central and nasal, mid-peripheral corneal thickness, subjective refraction, axial length and anterior chamber depth under cycloplegia, were evaluated pre- and immediately post-lens wear.

Results In the OK lens wearing (treated) eye, there was a significant reduction in myopia 1.04 ± 0.47 D ($p = 0.0001$), and a significant anterior corneal radius flattening (0.20 ± 0.11 mm; $p < 0.001$). Control eyes demonstrated no significant change in refraction nor in anterior corneal radius. Central corneal swelling occurred in both treated and control eyes but was significantly greater in treated eyes (treated: 57.56 ± 37.94 μm vs. controls 23.93 ± 12.58 μm, $p = 0.015$). The nasal mid-peripheral cornea swelled in both treated and controls, with no significant difference between the 2 groups. Anterior chamber depths shallowed significantly by 20.59 ± 24.39 μm ($p = 0.035$) in controls and 64.22 ± 34.00 μm ($p = 0.0004$) in treated eyes.

Conclusion Compared with conventional designs, ortho-k lenses create greater increases in corneal thickness and shallower anterior chamber depths. The results suggest that the central posterior cornea flattens more under OK than conventional lenses in the immediate phase following lens removal, the aetiology of which may include a mechanical component in addition to oedema.

■ 3453**Effect of intraoperative Mitomycin C (MMC) in LASEK refractive surgery on corneal endothelium cells**

PIZZAMIGLIO-MARTIN C (1), ORTEGA M (2), DE BENITO L (3)

SANCHEZ-PINA J (2), TELIS MA (3, 4)

(1) Cornea and ocular surface, Visum Hospital oftalmologico, Madrid

(2) Optometry, Visum Hospital oftalmologico, Madrid

(3) Cornea and refractive surgery, Visum Hospital oftalmologico, Madrid

(4) University Hospital, Alcala

Purpose To evaluate the possible toxicity of corneal endothelium by the use of MMC 0.02% applied during 30 seconds intraoperatively in LASEK.

Methods Prospective, single masked observer study. 24 patients (48 eyes) were divided in two groups: Group 1 (22 eyes) without MMC and Group 2 (26 eyes) with MMC application. Cells density was compared in both groups, preop and three months after surgery.

Results The mean preoperative age and mean refractive sphere were in Group 1 of 30.3 ± 1 years old, -2.3 ± 1.0 with a mean astigmatism of -0.6 ± 0.6 D. In Group 2 32.0 ± 0.9 years old, -6.4 ± 2.2 sphere and a mean astigmatism of -1.4 ± 0.1 D. The mean preoperative cells number were 2468.1 ± 269 in Group 2 and 2473.0 ± 341 in Group 2 preop. There was no statistically significant difference between cells density in both groups 3 months after surgery.

Conclusion MMC in concentrations and exposure time as used in this study doesn't seems to produce corneal endothelium toxicity, that compromises their function and integrity.

■ 3454 / 343**In vivo interferometric measurements of the tear film stability on soft contact lenses**

SZCZESNA DH (1), STENEVI U (2), KASPRZAK H (1)

(1) Institute of Physics, Wroclaw University of Technology, Wroclaw

(2) Department of Ophthalmology, Sahlgren University Hospital, Mölndal

Purpose The purpose of this study was to measure the stability of the precontact lens tear film on different types of soft contact lenses.

Methods We applied a non-invasive, interferometric method – Lateral Shearing Technique for *in vivo* investigating of the smoothness of external layer of the tear film and its stability (TFS) in time. Interferometry allows dynamic measurements of the TFS in real time by observation of the regularity of interference fringe pattern. Evaporation of tears, their instability and appearance of the breakups cause changes in fringe regularity. Fast Fourier Transform has been used for quantitative assessment of the fringe smoothness and the parameter M2 was introduced for quantitative description of the smoothness of the tear film. This parameter is lower for a smooth and higher for irregular surface of the tear film.

Results We examined three different groups of contact lenses: daily, two-weekly and monthly. All examined twenty subjects had no problems with the tear film. The stability of the tear film was examined before the contact lens wear, 5 minutes after contact lens morning application, the same day in the afternoon and two weeks later. In majority cases (69%) the tear film smoothness on the contact lens was directly correlated with the tear film quality on the cornea. The tear film is more stable between blinks on daily than on monthly contact lenses. The tear film is less stable at the beginning of the contact lenses wear and more stable in afternoon or after two weeks.

Conclusion The method is characterised by a high accuracy and sensitivity. Evident differences can be demonstrated in tear film quality on soft contact lenses.

■ 3455 / 344

Oxygen Transmission Characteristics of a Silicone Hydrogel Lens in Custom Parameters

GILES TG

CIBA Vision, Duluth

Purpose The introduction of silicone hydrogel lenses using high volume manufacturing has made this technology accessible to the majority of more than 89 million estimated contact lens wearers worldwide. There remains however an important sub-segment of the population for whom silicone hydrogel lenses are not available. This group consists of several million individuals who require extreme parameters, ranging from high plus and high minus powers to high cylinder powers and to designs which are flatter or steeper as well as larger or smaller than typical cast molded lens parameters.

Methods Silicone hydrogel prototype lenses have been produced in an 8.4 mm base curve and 14.0 mm diameter in powers of +16.00 DS, -16.00 DS, -3.00 DS and -3.00/-2.25 @ 090. Using a Lens Thickness Profiler, lens thickness across the entire surface of each lens was measured and the Dk/t calculated and plotted. This procedure was repeated with traditional hydrogel materials (phemofilcon A 38% water and methafilcon B 55% water) in the same power with similar parameters.

Results The Dk/t maps demonstrate the enhanced oxygen transmission profile for the silicone hydrogel material compared with traditional hydrogel materials. This is especially evident in the high plus and high minus designs, where lens thickness increases considerably.

Conclusion Modern silicone hydrogels have dramatically transformed the landscape of contact lens practice in the 21st century and clinical studies have shown fewer hypoxic signs among wearers of silicone hydrogel wearers. The availability of these materials in expanded, made-to-order parameters will allow practitioners to satisfy a previously unmet need among the patient population which stands to benefit the most from an enhanced oxygen profile.

■ 3456 / 345

Solutions, Contact Lenses & Wear: Then & Now

FRANKLIN VJ, TIGHE BJ

Biomaterials Research Unit, Aston University, Birmingham

Purpose Since the introduction of corneal contact lenses over fifty years ago, clinicians have sought & developed solutions, which promote or enhance lens comfort upon insertion & during wear. These have evolved from the relatively simple wetting solutions used with rigid polymethyl methacrylate to the highly sophisticated multi-purpose solutions used the current generation of contact lens materials. The underlying problem in the design of ophthalmic biomaterials continues to be that of ocular compatibility, although our understanding of the underlying biological issues involved has become progressively more sophisticated. Legislation, new cleaning regimes, contact lens materials & wear modalities all have had an impact on the perceived clinical performance & acceptability.

Methods This poster summaries some of the developmental issues & properties of the components of contact lens solutions & the impact of different wear modalities & contact lens materials on the use of such materials in the ocular environment, paying particular attention to the changes that have been seen in the last decade.

Results Taken together the properties of the solutions described provide a basis for understanding & interpreting the clinical behaviour of ophthalmic biomaterials used for contact lens applications, especially the solutions sensitivity observed with the new generation of silicone hydrogel lenses.

Conclusion Reintroduction of silicone hydrogels for contact lens applications & recent issues relating to compatibility with contact lens solutions has highlighted the complexity of ophthalmic biomaterials & the need to develop specific material & solution combinations for optimal ophthalmic performance.

■ 3457 / 346

Microfluidic Technology for Routine Tear Analysis

MANN A, TIGHE BJ

Biomaterials Research Unit, Birmingham

Purpose To study and obtain individual tear protein profiles by monitoring the effects of contact lens wear and factors affecting the normal ocular response and to assess the use of the contact lenses as a probe and means of sampling the tear film. The use of tears as a non-invasive diagnostic medium has great clinical potential.

Methods This work employed a fully automated chip based protein separation assay that is vastly superior and more convenient than comparable conventional gel electrophoresis methods. 4ml of tears were collected from a variety of patients in a series of self-controlled, clinically managed, studies. The tear envelope that is attached by surface forces to the contact lenses at the time of removal was also evaluated. All samples were assayed on a 2100 Agilent Bioanalyser with Protein Lab Chip' kits.

Results These studies investigated tear proteins in the range of 14-200kDa paying particular attention to the relative concentrations of lysozyme, lipocalin, sIgA, IgG and lactoferrin, in conjunction with overall tear electropherogram 'fingerprint'. Distinct protein compositional changes were observed between the lens and non-lens wearing eye, and the normal and compromised eye. In all 15 distinct peaks were detected, some of the peaks were found in only a small number of subjects or on a single occasion over the sampling period. Interestingly a peak at 16kDa which was not typically observed in normal basal tears, was common to the lens wearing tear and tear envelope.

Conclusion Laboratory technology is progressing towards miniaturisation and greater automation through the development of lab-on-a-chip platforms. These may in the future become part of routine clinical practice, thus incorporating diagnostic procedures into normal patient care.

■ 3458 / 347

Measurement of Frictional Characteristics of Contact Lenses

ROSS G, TIGHE BJ

Biomaterials Research Unit, Aston University, Birmingham

Purpose Biotribology is the study of the lubrication, friction & wear at biological interfaces. When a contact lens is placed on the eye, the surface of the contact lens will influence the motion between the eyelid & the surface of the eye & this influences lens comfort. This project is driven by the need to establish in vitro techniques for measurement of this important aspect of the interaction between contact lenses and the anterior eye.

Methods A high sensitivity tribometer has been identified & adapted for the study of contact lenses. The lens is placed on a convex mould, which slides against a moving substrate (which may be varied) in the optional presence of an appropriate lubricating solution. The resistance to motion is expressed in terms of the coefficient of friction. The whole apparatus sits on an air table to isolate it from vibration. Using this instrument, coefficients of friction down to 0.005 can be measured reproducibly.

Results Three distinct factors can be discerned that affect measurements made in vitro using this technique: bulk mechanical properties & the surface chemistry of the lens; nature of the lubricating layer; the hydration of the lens, & the deposition & subsequent degradation of tear components. Current silicone hydrogels show interesting individual 'friction fingerprints'.

Conclusion The technique shows clear material-dependant differences in the values of friction coefficient of different lens materials of unworn lenses & also changes in the frictional behaviour of lenses as a consequence of wear. It provides an additional tool in the study of the complex interactions that affect patient-to-patient & diurnal variations in comfort.

■ 3461**Role of inflammatory mediators in macula oedema**

BURKE J

The Eye Institute, Milwaukee

Purpose There is emerging evidence that for diseases that have macular edema as a common endpoint; multiple interacting factors appear to play significant roles. Pharmacotherapy with anti-vascular endothelial growth factor (VEGF) agents or anti-inflammatory agents such as steroids has shown clinical utility in managing several of these diseases and suggests that multiple pathways involving inflammatory mediators and VEGF are clearly important. To assist in elucidating mechanisms of various therapeutic approaches and to determine efficiency of therapy; primate models of macula edema are being employed.

Methods Two such models are: 1) Photothermal and photodynamic occlusion of a superior branch retinal vein in the arcade (BRVO); and 2) Intravitreal administration of hrVEGF165. In this latter model, the effect of DexDDS (700 ug), a polymer-based biodegradable system administered with a 22-gauge applicator that slowly releases the corticosteroid dexamethasone, was evaluated to examine the efficiency of anti-inflammatory therapy on inhibiting experimental macula edema. Six cynomolgus monkeys were used in the drug study, divided in 2 groups of 3 each and dosed unilaterally (OD) with either DexDDS or sham treatment. VEGF (1.25 ug in 50 ul) was delivered 1-2 mm anterior to the fovea using a 29 gauge needle at 1, 7 and 15 weeks following DexDDS or sham treatment.

Results Preliminary results from the BRVO model (n=4) show that the vessel closure was associated with transient hemispheric vasculopathy such as retinal vein dilation and tortuosity, focal hemorrhages and increases in retinal thickness. Intra- and subretinal fluid accumulation was more marked in the fovea (766 ± 255 um) at day 1 than in the superior hemisphere (340 ± 99 um). Edema was associated with a marked decline in retinal neurosensory function. In the VEGF model, the vasculopathy was similar, albeit global. Treatment with DexDDS prevented the vasculopathy throughout the 4 month experiment. The VEGF-induced foveal thickness was consistently greater in the sham group: 150 ± 9 um, 462 ± 181 um, 968 ± 270 um at the 2, 8 and 16 week timepoints, respectively. Foveal thickness remained unchanged over time in the DexDDS group: 133 – 137 um out to 4 months.

Conclusion Multiple pathways for macula edema suggest a variety of therapeutic opportunities. Animal models offer opportunities to evaluate benefits of various therapeutic strategies. Steroids represent therapies that work at several sites including blocking inflammatory processes and directly at the vasculature, and are very effective at reducing macula edema.

■ 3463**Clinical Characterization of DME***CLINHA-VAZ J (1, 2, 3)**(1) Ophthalmology Department of the University Hospital, Coimbra**(2) Centre of Ophthalmology of University of Coimbra, Coimbra**(3) AIBILI, Coimbra*

Purpose The clinical evaluation of macular edema has been characterized by its difficulty and subjectivity. We are now able to measure changes in retinal thickness and identify, using non-invasive instrumentation in a clinical setting, macular edema. Diabetic macular edema needs now to be identified regarding its type and distribution, its evolution, its pathophysiology, and degree of involvement of the central macular area. It is, therefore, necessary to consider 1) the distribution of the edema in the macula; 2) the evolution of the edema and its response to laser treatment; 3) the presence or absence of a situation of "open-barrier"; 4) the degree of involvement of the central fovea; 5) the extent of the macular edema; 6) the presence or absence of cysts in the retina demonstrating decreased retinal tissue pressure; 7) the presence or absence of vitreous traction and finally; 8) the presence of signs of ischemia such as loss of continuity of the capillary net surrounding the Foveal Avascular Zone or extensive areas of capillary closure.

■ 3462**Alternatives for Drug Delivery in DME**

KUPPERMANN BD

UC Irvine Department of Ophthalmology, Irvine

Purpose Laser photocoagulation is the standard of care in DME but the off-label use of intravitreal corticosteroids is becoming increasingly common. This presentation will discuss several novel intravitreal corticosteroid delivery systems currently being developed for use in DME.

Methods Review of prospective randomized clinical trials.

Results Posurdex® (sustained dexamethasone drug delivery system) consists of dexamethasone incorporated into a biodegradable polymer matrix. This small implant is placed into the vitreous via either a small incision or the use of an applicator (which produces a small, self-sealing wound). Dexamethasone is gradually released over several months while the polymer matrix gradually breaks down into inert compounds. In a 6-month, phase 2 study, a single Posurdex® treatment significantly decreased DME. Most adverse events were mild to moderate and occurred during the first week post-surgery. Retisert® is a sterile fluocinolone pellet that is inserted into the vitreous via a pars plana incision. Release of fluocinolone is sustained for approximately 30 months but the implant does not dissolve and remains in the eye unless removed. Retisert® is approved for the treatment of chronic posterior uveitis but not DME. Studies have shown that a single implant effectively reduces DME for several months but up to 60% of patients required IOP-lowering medication to control IOP and ~77.4% of patients developed serious cataracts (most requiring surgery). The Alimera fluocinolone implant and Surmodics triamcinolone helical coil are also in development for the treatment of DME.

Conclusion The variety of new drug delivery systems in development may offer the potential for sustained long-term therapy for this often chronic condition.

■ 3464**Clinical experience of different treatment modalities in DME**

BANDELLO F

Ophthalmology, Udine

Purpose Etiology of DME is still unclear, but current research efforts are focused on more effective therapies. A better understanding of the biochemical processes underlying DME has recently been fundamental in developing new treatment modalities. Under investigation are drugs that inhibit VEGF, PKC activation, and inflammatory mechanisms.

Methods Current international screening protocols and data from clinical experiences were reviewed.

Results The role of VEGF in DME has prompted the development of inhibitors such as pegaptanib, ranibizumab and bevacizumab. In clinical trials pegaptanib demonstrated a significant improvement in vision. Similarly, a trial evaluating the intravitreal humanised anti-VEGF antibody fragment ranibizumab is ongoing. Efficacy of bevacizumab has been recently demonstrated in small case series. The inhibition of PKC may play an important role in the progression of DME. Ruboxostaurin showed a non significant benefit on progression of DME but was useful in preventing visual loss in patients with DME at baseline, compared to placebo. Considering the important role of inflammation in the pathogenesis of DME, steroids could be a useful treatment option. Many papers reported the temporary efficacy of IVTA in reducing DME. Some Authors associated grid laser photocoagulation to IVTA to avoid recurrences of DME. One approach to achieving near constant intraocular steroids concentrations has been the development of sustained-release intraocular implants. An implantable intravitreal device that releases dexamethasone is being used in a Phase III clinical trial to evaluate the efficacy for DME.

Conclusion The results of large clinical trials of VEGF inhibitors, PCK inhibitors, TA and implantable steroids are eagerly awaited in the hope of reducing visual loss due to DME.

■ 4111**When is the right time to perform limbal grafting after severe ocular alkali burns**

GICQUEL JJ, NAVARRE R, DIGHIERO PL

Poitiers University Hospital / Ophthalmology Department, Poitiers

Purpose Even though the surgical techniques of limbal grafting are well standardized nowadays, the long term success rates either for auto or allografts are highly variable. In our experience, success rate don't seem correlated with the surgical method, but with the level of inflammation of the micro-environement in which the limbal graft is perform. We'll try to see how it is possible to better evaluate this micro-environment and when it is best to perform auto / or allo limbal graft in order to achieve the highest possible success rate.

■ 4112**Strengths and Weaknesses of the OOKP**

LIU C

Sussex Eye Hospital, Brighton

Purpose To list the strengths and weaknesses of the Osteo-odonto-keratoprosthesis (OOKP).

Methods Compilation based on published literature and author's personal experience.

Results Strengths. The OOKP is the only device which works in end-stage ocular surface disease. It has the best retention results of all devices for this disease group. There are warning symptoms and signs prior to extrusion, allowing pre-emptive action. This safety window is increased by regular imaging of OOKP lamina. It has good optics with a wide field of view. It also allows good visual acuity in cases without glaucoma and macular disease. The device is inexpensive with the optical cylinder costing around US\$200. Weaknesses. The surgery is complex and lengthy, and has a considerable learning curve. It is relatively irreversible as the iris, lens and a lot of the vitreous body are removed. There is difficulty with estimating intraocular pressure post-operatively. Topical anti-glaucoma medications cannot penetrate the buccal mucous membrane graft. There is no conjunctiva for trabeculectomy. There is necessarily significant oral damage. The resultant eye does not appear aesthetic.

Conclusion A thorough understanding of the strengths and weaknesses of a keratoprosthesis device assists with prudent patient selection and improved pre-operative counselling and consent. It also helps the surgical team to develop better protocols to take advantage of the strengths, and to get round the weaknesses of the device.

■ 4113**AlphaCor: An overview of current outcome data**

HICKS C

on behalf of the AlphaCor Users' Group, Nedlands

Purpose AlphaCor is an entirely synthetic alternative to donor tissue for the high risk case requiring corneal replacement. Every AlphaCor case has been entered into a database and followed anonymously prospectively through a compliant web-based database.

Results 88 surgeons reported data from 11 countries. To end April 2006, 331 devices had been implanted. Reported surgical techniques suggested a trend to implantation from within an existing failed graft wound rather than from a limbal incision, a procedure requiring approximately 45 minutes. Follow-up *in situ* was mean 1.4 years, median 1.2 years and maximum, 7.5 years, providing 450 patient-years' data. Patients had had mean 2.4 previous failed grafts (range 0 -13), with pathologies including trauma/chemical burn (23.0%), limbal stem cell failure including aniridia (9.1%) and ocular HSV (6.9%). Pre-operatively, glaucoma was reported present in 58.6%, with 26.9% having tubes *in situ*. Post-operatively, 0.3% required a tube. Post-operatively the probability of retention *in situ* at 6 months was 92%, at 12 months 81% and at 2 years 63%. Mean gain in BCVA was over 2 lines (range PL - 6/6) and mean post-op BCVA was not statistically significantly different from BCVA achieved from the most recent graft before it failed (paired samples t-test).

Conclusion AlphaCor provides an entirely synthetic and reversible alternative to donor tissue in high risk cases requiring corneal replacement. Surgical techniques and patient management and outcomes have advanced considerably during the life of the follow-up database.

■ 4114**The future of corneal bioengineering in the prevention or treatment of graft rejection**

BOURGES J-L (1, 2, 3)

(1) Ophthalmology, Hotel-Dieu Hospital, Paris

(2) Rene Descartes Paris 5 University, Paris

(3) INSERM U598, Cordeliers Institute, Paris

Purpose After penetrating keratoplasty (PK), alloimmune mediated corneal graft rejection is a well known risk factor of graft rejection recurrence by itself, but also remains the main cause of secondary endothelial failure and graft opacity. Results of recent researches help us to better understand the significant host or donor risk factors and mechanisms of rejection. To take advantage of such advances, various approaches have been proposed in the prevention or in the reversion of acute graft rejection and will be presented. Drug delivery systems or prodrugs as well as vectorisation devices have been designed to improve the efficacy of various active molecules. Laser or active immune therapies are also tested in this way.

■ 4121**Acute visual loss: Evaluation and differential diagnosis**

PURVIN V

Midwest Eye Institute, Indianapolis

Purpose The two most common causes of acute optic neuropathy with disc edema are optic neuritis (ON) and ischemic optic neuropathy (AION) which can usually be differentiated on clinical grounds. The most helpful features for distinguishing these two conditions are: age, presence of pain, pattern of visual loss and optic disc appearance. The diagnosis of acute optic neuritis can usually be confirmed with a good quality orbital MRI that includes gadolinium infusion and fat suppression. The prognosis for visual recovery in ON is excellent regardless of treatment. IV steroids are sometimes offered to decrease the chance of a repeat attack in at-risk individuals but such treatment does not affect visual outcome. In AION the most important task is differentiating the arteritic and non-arteritic forms. Helpful features include: systemic symptoms, laboratory testing, severity of visual loss, disc appearance (affected and fellow eyes), patient age and bilaterality. Patients with suspected Giant Cell Arteritis (GCA) should be treated emergently with high dose corticosteroids. ESR and CRP should be drawn prior to starting treatment but it is not appropriate or necessary to withhold steroids while temporal artery biopsy is pending. In patients with acute optic neuropathy and a normal optic disc, the main diagnostic considerations are retrobulbar optic neuritis (considerations the same as for ON above), posterior ischemic optic neuropathy (PION) and compressive or infiltrative processes. The retrobulbar optic nerve is relatively immune to ischemia due to atherosclerosis; the main consideration for patients with PION is GCA. Compressive etiologies include ophthalmic artery aneurysm and tumors, particularly pituitary apoplexy.

■ 4122**A dilated pupil: Neurologic or pharmacologic?**

BOSCHIA A

Neuroophthalmology, Brussels

Purpose Guidelines to work out a dilated pupils.

Methods The most common cause of asymmetric pupils is nonpathologic simple anisocoria. It is characterized by normal pupillary reaction with a little change of anisocoria under light and dark conditions. If the anisocoria is not physiologic, the first step is to work out which pupil is abnormal.

Results If anisocoria is greater in light, and diminishes in darkness, the dilated pupil is likely to be the abnormal, which is due to defective innervation of the iris sphincter muscle. Subsequently, faced with a pupil with a defective pupillary reaction to light without visual loss or oculomotor disorders, the following etiologies need to be considered: Adie syndrome, accidental pharmacological mydriasis, iris sphincter lesion (trauma, iritis, angle closure glaucoma). The next step should include careful analysis of the lid fissure and ocular motility, search for ptosis or ophthalmoplegia, followed by proptosis and visual loss. If the dilated pupil is associated to ptosis and some ophthalmoplegia, third nerve palsy should be suspected, but, in very rare instances, a dilated pupil is the only manifestation. In the presence of such a clinical picture, an aneurysm of the posterior communicating artery should be considered until proven otherwise. Lesions affecting the dorsal midbrain may also interfere with pupillary reactivity, with a light near dissociation. Usually oculomotor disturbances accompanied involvement of both pupils, although their size and light reactivity might be asymmetric. If visual loss or orbital signs are associated with a dilated pupil, a lesion located at the apex should be suspected.

Conclusion The clinical and diagnostic approach for each of these etiologies will be discussed through clinical cases presentations.

■ 4123**Sixth nerve palsy: When is it a sign of danger?**

MILEA D

University Hospital Copenhagen, Copenhagen

Purpose The most common cause of sixth nerve palsies is believed to be related to microvascular disease causing the so-called vasculopathic abducens palsy. In these typical cases in patients with vascular risk factors, the initial work-up is minimal, since other underlying causes are rarely found. In these vasculopathic sixth nerve palsies, the abduction deficit has usually a spontaneous favorable outcome within months. In rare circumstances a sixth nerve palsy can reveal an underlying visual or even life-threatening condition such as secondary raised intracranial pressure, compression from a tumor or aneurysm, Wernicke's encephalopathy, etc. An isolated abduction deficit can be also the presenting sign in giant cell arteritis. During the presentation, we will review the potentially visual or life-threatening conditions causing a sixth nerve palsy and the criteria allowing their early detection. We will also review a management algorithm (observation versus neuroimaging and/or other ancillary tests) depending on the clinical findings at presentation (patient's age, isolated versus non-isolated abducens deficit, unilateral versus bilateral deficit).

■ 4124**Visual loss from papilledema: Management and prognosis**

KAWASAKIA

Hôpital Ophtalmique Jules Gonin, Lausanne

Purpose Permanent visual loss is the chief morbidity associated with papilledema and in general, the risk of visual loss correlates with the severity of papilledema. Regular assessment of papilledema and optic nerve function, particularly with formal perimetry, is a critical aspect in managing patients with papilledema. Underlying conditions such as anemia, hypoxia or blood pressure problems which may aggravate or provoke optic nerve damage should be treated. Patients with mild visual field loss with a stable clinical course can be managed medically. Acetazolamide is the mainstay medication; furosemide can be used alternatively or additively. Management for papilledema becomes a more emergent situation when patients continue to have progressive deterioration of vision despite medical therapy or demonstrate moderate-to-severe visual field loss in one or both eyes at the time of presentation or have severe papilledema in the face of unstable blood pressures (for example, patients on dialysis). Acute temporizing measures include intravenous acetazolamide, intravenous steroids, repeated lumbar punctures or an externalized lumbar drain. The definitive treatment is surgical, either an optic nerve sheath fenestration or a CSF diversion procedure (lumboperitoneal or ventriculoperitoneal shunting). Both forms of surgery are highly efficacious in stabilizing and even improving optic nerve function post-operatively, although their relative disadvantages differ.

■ 4125

Radiation optic neuropathy: Can hyperbaric oxygen help?

BORRIUAT FX

Hôpital Ophthalmique Jules Gonin, Lausanne

Purpose To describe the clinical presentation, the neuroimaging results and the therapeutic options of radiation optic neuropathy (RON).

Methods RON is a dramatic, late and unpredictable complication of radiotherapy. Patients present with an acute painless visual loss resulting from radionecrosis of the anterior visual pathway, sometimes preceded by transient visual obscurations. Visual loss occurs after a median time of 13 months but can happen as early as 3 months and as late as 12 years after irradiation. Initial examination reveals a normal optic disc, except when radiation was delivered to the optic disc/retina (radiation papillopathy). Magnetic resonance imaging (MRI) reveals an enlargement of the optic nerve/chiasma enhancing with gadolinium. Bilateral involvement is frequent and visual prognosis is poor, final visual acuity being no light perception in 45% and ≤ 20/200 in 85% of cases. Risks for developing RON include a total dose > 50Gy, a fraction-dose > 200cGy, simultaneous chemotherapy, diabetes mellitus, and compressive lesions of the visual pathway. However, despite the use of a "safe dosage" of irradiation, some patients still develop RON. Visual loss from RON is almost always definitive and the search for an efficient preventive or curative therapy is still on.

Results Today, therapeutic options include corticosteroids, anticoagulants and hyperbaric oxygen (HBO). HBO delivered at 2.4 ATA (30 sessions, 100% O₂ for 90 minutes) has been shown to influence favourably the course of visual loss in some patients with RON.

Conclusion HBO is recommended in the treatment of selective cases of RON.

■ 4131

Clinical and pathological review of eyelids tumours

LASUDRY J

Clin. Univ., Hôp. Erasme, Brussels

Purpose The general management of eyelid tumours is based on two main considerations and apparently contradictory constraints. First, in case of malignancy, surgical removal has to be complete and with sufficient margin width, so as to reduce the risk of recurrence at the lowest possible. Second, eyelids are organs of reduced size and complex function, making desirable to limit the sacrifice of tissue to the minimum needed.

Methods To meet these requirements, the clinical evaluation is a critical and helpful step which guides the rest of the management. The vast majority of eyelid tumours are benign, and among these, most are cystic, or inflammatory, arising from the adnexae. The most common malignancies of the eyelids are the basal cell and the squamous cell carcinomas. Sebaceous gland carcinoma is rare, and often represents a diagnostic challenge. In case of equivocal clinical presentation, pre-operative biopsies may be useful to determine the nature and extend of the tumour, especially in unusual presentations.

Results When the diagnosis is straightforward, selected eyelid malignancies may benefit from per-operative (extemporaneous) pathological evaluation of the margins of resection. Frozen section and Mohs's micrographic evaluation are considered techniques of choice for basal cell carcinoma, squamous cell carcinoma, and carcinomas from various adnexal origins. Sebaceous gland carcinoma and melanoma are more difficult to monitor with frozen sections. Rather, their evaluation may benefit from fixed tissue techniques, delaying thus the reconstruction step.

Conclusion Reconstruction is considered only after the excision step has been ascertained to fulfil the rules of carcinologic surgery. Clinical evaluation contributes to elaborate the desirable endpoint of the whole procedure.

■ 4132

Basal cell carcinoma of the eyelid in Finland during 1953-97

SAARI KM, PAAVILAINEN V

Dept of Ophthalmology, University of Turku, Turku

Purpose To study the incidence of basal cell carcinoma (BCC) of the eyelid in Finland.

Methods We studied 6241 cases of BCC of the eyelid reported to the nationwide Finnish Cancer Registry during 1953-97. We determined the age- and sex-specific incidence rates and overall rates adjusted for age to the world standard population, and social class- and occupation-specific standardized incidence ratios, with the total Finnish population as reference.

Results The incidence rates of BCC of the eyelid varied between 0.7 and 3.0 per 100 000 person-years in men and between 0.5 and 2.8 per 100 000 person-years in women during the study period. The age-adjusted incidence rates of BCC of the eyelid increased during 1953-87 ($p<0.0001$). The incidence of BCC of the eyelid rose significantly with age. There were no significant differences in standardized incidence ratios (SIRs) for BCC of the eyelid between different social class and occupation categories.

Conclusion Age-adjusted incidence rates showed that BCC of the eyelid was more twice as frequent during 1978-97 than before 1968. Ageing may partly explain the increased incidence of BCC of the eyelid.

■ 4133

Clinical and histopathological characteristics of recurrent BCC of the eyelids

ROSNER M

Tel Aviv University, Sheba Medical Center, Tel Hashomer

Purpose Basal cell carcinoma (BCC) is the most common skin cancer of the eyelids. The recurrence rate of treated BCC of the eyelid averages about 8.5%. The purpose of this study was to find out any demographic, clinical or histopathologic features that are typical of recurrent BCC of the eyelids.

Methods The charts of 17 patients with recurrent BCCs and 67 patients with primary BCC of the eyelids were reviewed for clinical information. All the tissue specimens of BCCs excised from these patients were reviewed. The gender and age of the patients, and the size, location and histopathologic features of the recurrent and the primary lesions were compared.

Results Recurrent and primary BCCs of the eyelids were similar in clinical and histopathologic presentation. The recurrent group included 53% males as compared to 61% males in the primary tumors group. The mean age was similar. Large lesions constituted 32% of the recurrent and 16% of the primary group. The location of tumors was the same with the lower lid being the most common. In both groups the solid differentiation was the most common and in descending prevalence were the morphea and mixed.

Conclusion The finding that recurrent and primary BCCs of the eyelids were similar in clinical and histopathologic presentation, suggests that incomplete excision may indeed be the main cause of recurrence, and not a special behavior of the tumor. The infiltrating pattern was not found to be more prevalent in recurrent tumors. Even in cases with BCC showing nodular presentation, with clinically obvious border, careful examination of the surgical margins should be performed in order to excise the tumor completely.

■ 4151**Perimetric Performance of Artificial Neural Networks based on Pattern Deviation for Glaucoma Diagnosis**

BIZIOS D, BENGTSSON B, HEIJL A

Department of Clinical Sciences, Ophthalmology, UMAS, Lund University, Malmö

Purpose To investigate the effects of perimetric input data on the performance of a classifier based on Artificial Neural Networks (ANNs), evaluate this trained classifier on an independent patient material, and compare its diagnostic accuracy to that of other conventional algorithms.

Methods SITA-Standard visual fields from 213 healthy subjects, 127 glaucoma patients, 41 patients with cataract and 68 patients with concomitant glaucoma and cataract formed the initial training dataset. The different input types were the raw threshold sensitivity values, Statpac Total and Pattern Deviation values, and probability scores based on Total and Pattern Deviation probability maps. The best performing ANN was then tested on visual fields from 100 glaucoma patients and 116 healthy individuals, and its accuracy was compared to that of the Glaucoma Hemifield Test (GHT), the Pattern Standard Deviation (PSD) index at $p<5\%$ and $p<1\%$, and to a technique based on recognizing clusters of significantly depressed test points.

Results Results were much better when Pattern Deviation probability scores (area under ROC 0.988) were used as input rather than raw threshold sensitivity values. On the new patient material, this ANN achieved a sensitivity of 93% at 94% specificity (area under ROC 0.984). Its diagnostic accuracy (93.5%) was slightly to considerably better, compared to the conventional algorithms.

Conclusion The ANN based upon Pattern Deviations provided the best results. Its high diagnostic performance, further confirmed in an independent sample, indicate the potential of ANNs as clinical glaucoma diagnostic tools.

■ 4152**Search for an optimal combination of structural and functional methods for the diagnosis and follow-up of glaucoma**

SKORKOVSKA S (1), MICHALEK J (2)

(1) Clinic of Eye Diseases and Optometry Masaryk University, Brno

(2) Dept. of Applied Maths and Computer Science Masaryk University, Brno

Purpose To evaluate which of the structural and of the functional diagnostic methods can give the best differentiation between healthy and glaucomatous eyes.

Methods Only one eye of 40 healthy individuals and 40 age-matched patients with primary open angle glaucoma were included in the prospective study. Structural evaluations were conducted with the Heidelberg Retina Tomograph and semiquantitative retinal nerve fiber layer (RNFL) scoring according to Airaksinen method. The functional parameters were reviewed by means of standard white-on-white (W/W) and blue-on-yellow (B/Y) perimetry. The difference between measured parameters of the healthy group and glaucomatous group was tested by Mann-Whitney test. The classification and regression trees methodology was used for identifying the parameters with the highest classification ability. Then the discriminant analysis was performed to find the best discriminant function of parameters for each diagnostic method (HRT, RNFL loss, W/W and B/Y perimetry). The accuracy of discrimination was described and visualized by the ROC curves. The smooth estimate of the ROC curve for binormal model has been used.

Results They were statistically significant differences between both groups in the level of intraocular pressure, mean sensitivity and mean defect of visual field of W/W perimetry and scoring of RNFL loss. The parameters with the highest diagnostic ability obtained by classification trees methodology were: scoring of RNFL loss, the level of IOP and mean defect of W/W perimetry.

Conclusion Evaluation of RNFL loss and IOP were found to be the most useful diagnostic methods for discrimination of healthy and glaucomatous eyes by means of classification trees and ROC curves.

■ 4153**Reproducibility of optic disc examination by HRT III**

MIGLIORI S (1), GUARESCHI M (1), BERTUZZI F (1), DI MATTEO F (1), RULLI E (2)

(1) Clinica Oculistica, Policlinico di Monza, Università Milano Bicocca, Monza
(2) Istituto Mario Negri, Milano

Purpose To assess the reproducibility of the measurement of stereometric parameters of the optic disc by means of the Heidelberg Retina Tomograph III.

Methods HRT examination was repeated three times by 2 different observers (6 images) on the same day on one eye of each of 17 subjects. The image with the lowest standard deviation was chosen (2 images, one for each observer). The two observers independently traced the contour line on the 2 chosen images. For the intra-observer inter-image evaluation, the results based on the contour lines traced by each observer on the two images were taken into account. For the inter-observer intra-image evaluation, the analysis took into account the results based on the contour lines traced by the two observers on the same images. For the inter-observer inter-image evaluation, the analysis took into account the results based on the contour lines traced by the two observers on the images taken by each of them. The analysis of reproducibility of the Glaucoma Probability Score (GPS) took into account the results automatically provided by the instrument for each of the chosen images. Reproducibility was calculated by means of the intra-class correlation coefficient (ICC).

Results The ICC ranged between 0.31 and 0.94 for intra-observer inter-image evaluation, between 0.71 and 0.96 for inter-observer intra-image evaluation, and between 0.41 and 0.85 for the inter-observer inter-image evaluation. As far as the analysis of reproducibility of the GPS is concerned, the ICC ranged between 0.68 and 0.70 depending on the different sectors of the disc.

Conclusion Measurement of optic disc stereometric parameters by HRT III is fairly to almost perfectly reproducible (0.41 \leq ICC \leq 0.99). GPS showed a substantial degree of reproducibility.

■ 4154**Can new version 3 software improve diagnostic ability of Heidelberg Retina Tomograph parameters?**

FERRERAS A (1), PAJARIN AB (2), BORQUE E (1), PUEYO V (1), POLO V (1), LARROSA JM (1), PABLO LE (1), HONRUBIA FM (1)

(1) Ophthalmology, Miguel Servet University Hospital, Zaragoza
(2) Primary Care, Zaragoza

Purpose To compare the diagnostic ability of the optic nerve head parameters obtained by using the Heidelberg Retina Tomograph (HRT) version 3 with those of HRT II.

Methods 71 normal eyes and 75 glaucoma subjects (altered standard automated perimetry and glaucomatous optic nerve head appearance) were included. All of them underwent optic disc topography by using the HRT II and the HRT3. The receiver operating characteristic curves were plotted for all global stereometric parameters of both HRT versions.

Results RB, FSM, vertical cup/disk ratio and the cup shape measure showed the greater areas under the ROC curve for HRT II, and RB, FSM, rim volume and vertical cup/disk ratio for HRT3. RB yielded a 74% sensitivity and 93.7% specificity (at a cut-off point of ≤ 0.62) for both software versions. No significant differences were found between equivalent parameters of HRT II and HRT3.

Conclusion HRT II and HRT3 global parameters presented similar diagnostic ability to discriminate between healthy and glaucomatous eyes. RB discriminant function showed the higher sensitivity-specificity balance.

■ 4155 / 435

Influence of Central Corneal Thickness in pre-perimetric glaucoma subjects defined by means of HRT II, OCT and GDx VCC

BORQUE E, LARROSA JM, POLO V, FERRERAS A, MAYORAL F, HONRUBIA FM
Ophthalmology Dpt, Miguel Servet University Hospital, Zaragoza

Purpose To compare the corneal ultrasonic pachymetry results between normal and pre-perimetric glaucoma subjects in different age groups.

Methods 61 normal eyes (IOP<21mmHg, with normal standard automated perimetry -SAP- and no glaucomatous appearance of the optic nerve head) and three different pre-perimetric glaucoma subset of patients, were included. Pre- perimetric glaucoma groups were age matched with normal group, and presented IOP>21, normal SAP and one of the following criteria: MRA of the HRT II "borderline" or "outside normal limits" (53 eyes), average thickness of the OCT with $p<0.05$ (27 eyes) or NFI of the GDx VCC >29 (23 eyes). Only one eye was randomly chosen for the study. All of them underwent corneal ultrasonic pachymetry (DGH 500). Mean central corneal thickness (CCT) were compared between normal and pre-perimetric glaucoma subjects in different age groups.

Results In general terms, no significant differences were found in the CCT between healthy subjects and the pre-perimetric glaucoma eyes defined by the three structural tests in any of the age groups. No lineal relation was found between IOP and CCT.

Conclusion There is not a greater risk to develop glaucomatous damage in subjects with thinner CCT. It is complex to adjust IOP depending on CCT as the relationship between them is not lineal.

■ 4156 / 430

Impact of Neuroretinal Rim Measurements as Predictive Factor for the Development of Glaucoma in Patients with Ocular Hypertension

LAEMMER R (1), MARTUS P (2), MARDIN CY (1)

(1) Department of Ophthalmology, University of Erlangen-Nuremberg, Erlangen

(2) Institute of Biostatistics and Clinical Epidemiology, Charité University Medicine, Berlin

Purpose The purpose of this study was to investigate morphologic predictive factors for the development of glaucoma in patients with ocular hypertension (OHT).

Methods Two hundred eighteen eyes of 109 patients with OHT (normal visual field, normal optic disc appearance, IOP > 21 mmHg) and an observation period of more than 5 years were included in the study. All patients received an annual, detailed, standardized glaucoma examination. 21 eyes of 17 patients had marked neuroretinal rim loss and development of glaucomatous optic disc atrophy during follow up, identified by masked comparative evaluation of stereographic optic disc slides by two experienced observers independently from each other. 13 eyes of 10 patients from this subgroup developed reproducible visual field defects. Standard HRT-parameter (global and 4 sectors) from baseline examination were compared between stable and progressive patients.

Results Significant differences ($p<0.01$, Mann-Whitney-U test) between both groups were found for most of the investigated parameters. In the progressive OHT group rim area and rim volume were significant lower. Also mean and peak height contour were significant lower in the progressive OHT group. Results were similar for global data as well as for sector analysis.

Conclusion Patients with OHT and conversion to early glaucoma during follow up had already at baseline a lower neuroretinal rim area and rim volume, detected by scanning laser tomography, if compared with the non-progressive group. This supports recent studies showing that low neuroretinal rim area and volume in healthy discs maybe a risk factor for the development of glaucoma.

■ 4157 / 431

Inadequate anterior segment compensation is not a major cause of false-negative GDx Nerve Fiber Analyzer test results

MUSKENS RPHM (1, 2), HEEG GP (1), JANSONILIS NM (1, 2)

(1) University Medical Center Groningen, University of Groningen, Groningen

(2) Department of Epidemiology & Biostatistics, Erasmus Medical Center, Rotterdam

Purpose To investigate the influence of inadequate anterior segment compensation on the screening performance of the GDx Nerve Fiber Analyzer with fixed anterior segment compensation(GDx).

Methods The GDx-parameter Ellipse Average was measured in the macula as a measure of the inadequacy of anterior segment compensation in 29 false-negative glaucoma cases (i.e. glaucoma patients with a normal GDx test result, defined as the Number <30) and 105 controls.

Results Macula Ellipse Average was $57 \pm 11 \mu\text{m}$ (mean \pm SD) in the false-negative glaucoma patients and $62 \pm 13 \mu\text{m}$ in the controls (t-test: $P=0.06$).

Conclusion Inadequate anterior segment compensation does not seem to be the major cause of false-negative GDx test results.

■ 4161

VEGF Pharmacology and Role in Ocular Angiogenesis

SCHLINGEMANN RO
AMC Ophthalmology, Amsterdam

ABSTRACT NOT PROVIDED

■ 4162

Triggers of VEGF in the eye

KVANTTA A

Department of Clinical Neuroscience, Section of Ophthalmology and Vision, Karolinska Institutet, S:t Eriks Eye Hospital, Stockholm

Purpose Ocular neovascularization may affect the retina, choroid and cornea and is commonly seen in some of our most common sight-threatening eye diseases including diabetic retinopathy, age-related macular degeneration and trachoma. Strong experimental and clinical evidence links vascular endothelial growth factor (VEGF) to the pathogenesis of ocular neovascularization and anti-VEGF strategies have emerged as a therapeutic option. The regulation of VEGF is complex and involves both oxygen- and inflammation-dependent components. This presentation will give an overview of our current knowledge on how VEGF is regulated in the normal eye and in pathologic ocular conditions.

■ 4163

Mouse models for diabetic retinopathy and age-related macular

BOSCH F
-, Barcelona

ABSTRACT NOT PROVIDED

■ 4164

Role of VEGF isoforms in the eye

STALMANS I (1, 2, 3), CARMELIET P (3)

(1) Dept of Ophthalmology, University Hospital, Leuven

(2) Laboratory of Ophthalmology, Catholic University, Leuven

(3) Center for Transgene Technology and Gene Transfer, Catholic University, Leuven

Purpose The role of VEGF-A in ocular neovascularisation and is well established. The specific role of the different VEGF-isoforms, however, is largely unknown.

Methods To study the differential role of the three major isoforms, three transgenic mice were generated that each express only one of the VEGF-isoforms. Physiological retinal vascular development was assessed in these mice using wholemount immunohistochemical and *in situ* hybridization techniques to visualize astrocytes, endothelial cells and pericytes.

Results Astrocyte migration preceding vascular outgrowth was normal in all three transgenic mice. Mice that only express the VEGF164 isoform showed normal vascular development. However, abnormal arterial outgrowth was observed in both mice that lack the VEGF164 isoform. The mice that only express VEGF188 exhibited a particular phenotype with persisting hyaloid vessels that dived into the retina and compensated for the lacking retinal arterioles. In the mice that only express the VEGF120 isoform, an immature vascular bed consisting of dilated and tortuous vessels were observed. These vessels were leaky despite the presence of a normal number of pericytes. Neuropilin was shown to be predominantly expressed along the retinal arterioles.

Conclusion The different VEGF isoforms exhibit distinct functions in physiological retinal angiogenesis. VEGF164 is sufficient to induce normal retinal vessels and seems to play a crucial role in retinal arterial outgrowth, possibly through the VEGF164-specific receptor Neuropilin-1. VEGF120, on the other hand, induces immature and leaky vessels. These findings, as well as other studies suggesting the need for minimal VEGF levels to avoid neurodegeneration and/or vascular pruning (regression of normal vessels), will be discussed.

■ 4165

Anti-VEGF from laboratory to clinics

SCHMIDT-ERFURTH U
Dept. of Ophthalmology, Lubeck

ABSTRACT NOT PROVIDED

■ 4211

Implatation studies of cross-linked collagen tissue substitutes into pig corneas - An update

FAGERHOLM P (1), LIU W (2), GAN L (1), GRIFFITH M (2)

(1) Dept of Ophthalmology, University Hospital, Linköping

(2) The University of Ottawa Eye Institute, Ottawa, Ontario

Purpose To determine the safety of tissue substitute materials implanted into corneas and to determine the efficacy in allowing for restoration of corneal innervation.

Methods 6.75 mm diameter, 500 µm thick pieces of 10% collagen – EDS/NHS were implanted into Göttingen minipig corneas by lamellar keratoplasty. No post-operative steroids were given. A clinical investigation was performed including slit-lamp examination, IOP measurements and confocal microscopy before harvesting the corneas. The optical properties of the specimen were examined before preparing them for light and transmission electron microscopy (TEM).

Results With a few exceptions the corneas were clear with no detectable haze as seen in the slit lamp. The transmission of light was of the same magnitude as the control corneas. 2 % of the light was scattered in the control corneas and 5-6 % in the operated corneas. This difference was not noticeable in the slit lamp. In vivo confocal microscopy showed nerve regeneration in the grafts, which was confirmed in TEM. Light microscopy showed a homogenous stroma in the grafted areas and the epithelium covering the graft was normal or slightly hyperplastic.

Conclusion The cross-linked collagen tissue substitutes engrafted well into the host cornea as shown clinically and histopathologically. After 12 months the grafted corneas were transparent. Both confocal microscopy and TEM confirmed nerve regeneration into the graft. Early results on on-going studies evaluating different types of human collagen as well as the influence of postoperative topical steroids will be presented.

■ 4212

Results from the Multi-Center Boston KPro Study Group

BELIN MWB (1, 2), ZERBE BZ (1)

(1) Albany Medical College Dept of Ophthalmology, Albany, NY

(2) Cornea Consultants of Albany, Albany, NY

Purpose To report the current results from the Multi-Center Boston KPro Study Group.(INVITED PAPER)

Methods 70 pre, intra, and postoperative parameters were collected from 19 sites since 1/1/03 to a central data collection site in an ongoing prospective study.

Results 158 procedures in 153 eyes of 148 patients since Jan. 1, 2003 were analyzed. Common preoperative diagnoses were repeated graft failure in 80 eyes (avg. prior grafts 2.3), bullous keratopathy (22), chemical injury (21), and HSV keratitis (9). Preoperative BCVA were < 20/200 in 94.9%. Postoperative BCVA improved to > 20/200 in 54%. Eyes > 1 year postoperative (39) obtained BCVA > 20/200 in 51% and > 20/60 in 37%. One year graft retention was 94.9%. Severe visual loss was usually secondary to co-morbidity (retina & glaucoma).

Conclusion The Boston KPro represents a viable alternative for patients with a poor prognosis for standard PK. Our initial results compare very favorably to previously published data on other types of keratoprosthesis.

■ 4213

Towards a synthetic OOKP support frame

VIITALA R (1), FRANKLIN VJ (1), TIGHE BJ (1), LIU C (2), LLOYD AW (3)

(1) Biomaterials Research Unit, Aston University, Birmingham

(2) Sussex Eye Hospital, Brighton

(3) Faculty of Science & Engineering, University of Brighton, Brighton

Purpose In OOKP surgery human tooth and bone are used as a support frame for the prosthesis, which makes the clinical use of OOKP very demanding and expensive. More recently degradation of the support frame in vivo has been reported which causes clinical problems. The aim of this project is establish an in vitro test to evaluate different commercial bioceramics which could be used to replace tooth and bone structure in OOKP.

Methods We have developed an in vitro test which measures the degradation of potential support frame matrixes in artificial dissolution medium. Artificial dissolution medium mimics the inorganic part of aqueous humour containing Na⁺, K⁺, Cl⁻, HCO₃⁻ and PO₄³⁻ and is buffered to pH=5.0 and pH=7.4. Dissolution of following bioceramics is done at temperatures 37 °C and 60 °C: calcium carbonate (Biocoral), hydroxyapatite (HAP), HAP 65% / tricalcium phosphate 35% (Biceram) and human tooth. The release of calcium and phosphate is measured and degradation rates for bioceramics calculated.

Results The degradation of ceramics was extremely slow at pH=7.0 and there was no difference between the ceramics during the first 6 weeks, whereas the degradation at pH 5.0 was much greater. All studied ceramics dissolved at pH=5.0 and the rate was fastest for Biocoral.

Conclusion This assay will provide a predicted method for assessment of in vivo dissolution behaviour of novel bioceramics as synthetic OOKP candidates in comparison to existing tooth and bone OOKP support frame.

■ 4214

State of the art of human corneal endothelial cell proliferation

THURET G, ZHAO M, GAIN P

Ophthalmology department, EA 3063 'Cell survival and adhesion in cancers and grafts', Faculty of medicine, Saint-Etienne

Purpose Human corneal endothelial cells have a well known particular proliferative status in adults with an apparent quiescence. They have been described, ten years ago, as blocked in the early G1-phase of the cell cycle and consequently capable of division under certain experimental conditions. The latest knowledges about the molecular events that regulates that arrest and those that could be modulated to trigger and control the re-proliferation will be discussed: modern molecular biology techniques like genomic and proteomic study could give us keys to better understand what could be the tomorrow therapeutic targets to either treat early stages of Fuchs dystrophy or secondary endothelial deficiencies, or improve graft quality during eye banking by increasing the endothelial cell density.

■ 4221

VEGF isoforms in ocular neovascularisation

BATES D

*Microvascular Research Laboratories, Department of Physiology, University of Bristol, Bristol***ABSTRACT NOT PROVIDED****■ 4222 Angiopoietins in the retina: physiological and pathological functions**

HAMMES HP

5th Medical Department, Mannheim

Purpose Diabetic retinopathy is characterized by an early loss of pericytes and progressive vascular occlusion. Vascular regression and pericyte recruitment are the domains of the angiopoietin/Tie system. Its regulation under hyperglycemic conditions, and its role in incipient diabetic retinopathy is uncertain. We studied the expression of the angiopoietins in cell cultures and in diabetic animals and used mice with heterozygous Ang-2 deficiency to assess its contribution to early retinopathy.

Methods The regulation of angiopoietin-2 (Ang-2) was studied in endothelial cells and Müller cells in normal and high glucose. The expression of Ang-1 and -2 was assessed in diabetic rats in association with retinal pericyte loss using morphometry of retinal digest preparations. Recombinant Ang-2 was injected intravitreally in non-diabetic rats to assess the outcome on pericytes. Pericyte loss and the retinal damage was analyzed in mice with heterozygous Ang-2 deficiency.

Results Exposure of both, endothelial cells and Müller cells to high ambient glucose concentrations led to an increase in Ang-2 transcription mediated by a glucose sensitive element in the Ang-2 promoter. In the early diabetic retina, Ang-2 upregulation preceded the onset of pericyte loss. This loss was prevented in the heterozygous Ang-2 deficient mice, together with a partial prevention of vessel regression. Pericyte loss was inducible by intravitreal injection of recombinant Ang-2 in non-diabetic rats.

Conclusion The data support the hypothesis that the Ang-Tie system plays an important role in hyperglycemic pericyte loss and in incipient diabetic retinopathy.

■ 4223

Is the Ephrin/Eph system essential for physiological and pathological retinal vascularization?

AGOSTINI HT

Department of Ophthalmology, Freiburg

Purpose The ephrin ligands and their Eph receptors are membrane bound and interact upon cell-to-cell contact. Bidirectional signalling is an essential feature of this receptor-ligand system and has best been studied in axon guidance for retino-tectal projection. A total of 9 ephrins and 16 Ephs are divided into group A and B. Some of them were found to be also involved in vascular development. EphrinB2 is expressed on arteries, while EphB4 is expressed on veins, mice lacking these genes show severe defects of their vasculature at an early embryonic stage.

Methods The investigations of ephrin/Ephs in ophthalmic disease from different groups are mainly based on immunohistological and molecular biological findings from human pathological tissue or retinal endothelial cell cultures, animal models of oxygen or laser induced angiogenesis and manipulation experiments with monomeric or dimeric EphB4 and ephrin B2.

Results In the murine model of oxygen induced retinopathy, the expression of ephrin B2 and EphB4 differs under hyperoxic and hypoxic conditions. It is possible to manipulate angioproliferation with monomeric and dimeric ephrinB2 and EphB4. However, there are controversial reports on the effect of dimeric EphB4.

Conclusion There is evidence that the ephrin/Eph ligand-receptor system is involved in proliferative eye disease and could serve as a therapeutic target. Controversial findings about the effects of monomeric and dimeric isoforms will be discussed on the background of different experimental models and findings in human disease.

■ 4224

Mechanisms underlying the angio-fibrotic switch in CNV and proliferative retinopathies

SCHLINGEMANN RO

*AMC Ophthalmology, Amsterdam***ABSTRACT NOT PROVIDED**

■ 4225

The dual role of macrophages in inflammatory lymphangiogenesis

CURSIEFEN C

Department of Ophthalmology, University of Erlangen-Nürnberg, Erlangen

Purpose To demonstrate the essential, dual role macrophages play in inflammatory forms of angiogenesis and lymphangiogenesis.

Methods Review of own and published research.

Results Macrophages play an essential and dual role in inflammation-associated forms of neovascularization. Using the cornea as an in-vivo model system we can demonstrate that macrophages not only release angiogenic and lymphangiogenic growth factors but also physically integrate into newly formed lymphatic vessels.

Conclusion Macrophages contribute by several mechanisms to the formation of new blood and lymphatic vessels. Novel antiangiogenic treatment strategies can be based on targeting macrophages.

■ 4231

Measurement of depth of basal cell carcinomas using pulsed ultrasound and its possible use in the diagnosis and treatment of tumors of the eyelids

ALLANE, LONCASTER J, SHERIDAN L, LEACH C
Christie Hospital, Manchester

Purpose We employ an Episcan 1-200 supplied by Longport International with a 32 MHz probe. The most important prognostic factor in the management of skin tumours is tumour thickness. Pulsed ultrasound provides an accurate assessment of tumour thickness to within a fraction of a millimetre. Ultrasound is preferable to needle biopsy assessment in that it is non invasive, takes a few minutes to perform with an immediate result. It measures the maximum tumour thickness. There is no subsequent scar formation from the scan as there may well be after a needle biopsy. A scan will demonstrate tumour thickness down to 0.2 mm. If a needle biopsy is considered to be necessary a scan will demonstrate the optimum site of maximum tumour thickness as the site for the biopsy.

Methods Practical benefits of a scan pre-treatment: Assessment of tumour boundaries including thickness to facilitate surgery. Decide on treatment modality - may be suitable for photodynamis therapy or Efudix. Decide on number of PDT sessions required. If XRT used decide on energy required. Assessment of response to treatment: Residual disease or recurrence. Will identify small tumour residue. May help to differentiate benign from malignant lesions. Identifies depth of laser fibre if this is inserted to treat thick lesions with PDT. Asses thickness of malignant melanomas so that sentinel node biopsy if indicated this can be done under the same anaesthetic as the primary excision.

Conclusion Audit and research: Response to new techniques or new agents. Accurate knowledge of tumour resolution combined with preliminary tumour thickness is essential if meaningful statistical analysis is to be achieved.

■ 4232

Sebaceous gland carcinoma of the eyelids - clinically and pathologically challenging cases

MUQIT MMK
Leeds Teaching Hospitals NHS Trust, Leeds

Purpose Sebaceous gland carcinoma (SGC) is the second most common malignancy of the eyelid. It is slow growing and mimics several benign conditions, with the clinical appearances seldom pathognomonic. Histopathological diagnosis remains challenging for hospital pathology services. Mortality rates from primary carcinoma may be as high as 40%.

Methods A clinicopathological case presentation format is used to highlight the diagnostic challenges in the management of eyelid SGC. It is important to demonstrate sebaceous differentiation within the group of eyelid neoplasms. Immunohistochemical staining using anti-adipocytic antibodies is described. This may be a useful ancillary technique for the demonstration of lipid in SGC that may be applied to paraffin sections.

Results Intraepithelial spread and masquerade presentations may delay the diagnosis. Recent immunohistochemical techniques demonstrating lipid within the cell cytoplasm are described. The surgical approach to eyelid SGC is aimed at reducing tumour recurrence and preserving eyelid function. The results of radical surgical technique and Mohs surgery are demonstrated.

Conclusion Astute clinical suspicion, early diagnosis, accurate histopathological diagnosis and aggressive surgical technique are important factors for reducing the mortality from eyelid SGC. The survival rate for eyelid SGC may be more favourable than previously reported.

■ 4233

Argon laser for the treatment of benign eyelids tumors

MOURIAUX F
Service d'ophtalmologie CHU, Caen

Purpose The ophthalmologists are able to differentiate a benign lesions from a malignant lesion of the eyelids at the slit-examination. The laser is suitable for use by ophthalmologists even if there are not surgeons. The lesions are localised and the treatment can be performed precisely to maintain adequate lid function and cosmetic.

Methods The patient is comfortably seated at the slit-lamp laser after local infiltration of 1% or 2% lidocaïne-adrenaline as well as topical instillation of Oxybuprocaine hydrochloride. A plastic shield is then placed under the lid to protect the eye from inadvertent injury. Laser parameters were from 400-1000 mW power, 2 sec time at continuous exposure with a spot size of 100µm at 400µm. After laser, antibiotic ointment is instilled twice daily for 5 days. Each patients was reviewed 8-10 days after the treatment.

Results Recurrence of the lesions are rare and require surgical removal with histopathology. The complications are rare. Potential complications include change of the skin texture and hypopigmentation. The histopathology is impossible if the treatment has burned the lesion. The histopathology may be difficult to interpret if the laser has remove the lesion.

Conclusion Argon laser therapy could be a therapy for some benign eyelid lesions

■ 4241

Effects of ET-1 and NO in an experimental model of rabbit eye

DELGADO E (1), MARQUES-NEVES C (2), ROCHA I (2), SALES-LUIS J (1),

SILVA-CARVALHO L (2)

(1) Clinics, Lisbon

(2) Physiology, Lisbon

Purpose A model of isolated rabbit eye was designed to help clarifying the effects of ET-1 and NO as a local regulatory mechanism of ocular circulation.

Methods Rabbit external ophthalmic arteries (n=24) in a head-mounted preparation were cannulated and concentration-response curves to intraarterial injections of ET-1 (n=6), to an intravitreal injection before an intraarterial injection of ET-1 (n=6) and to three intraarterial growing concentrations of L-Arg (n=6) and L-NAME (n=6) were obtained. Data was studied using paired t-test and wavelet transform.

Results Without any drug administration intrinsic vasomotricity was observed in the 24 rabbit models. ET-1 induced a strong and long lasting vasoconstrictor tone. Vasomotricity became more evident, showing a higher frequency and shorter amplitude of the oscillations. ET-1 intravitreal injection produced no significant changes. L-Arg and L-NAME elicited dose-dependent vasodilation and vasoconstriction, respectively. With L-Arg both frequency and amplitude of the oscillations decreased; with L-NAME the frequency of the oscillations increased but their amplitude decreased.

Conclusion Intraarterial ET-1 had a strong vasoconstricting effect and caused a faster rate of pulsatility. NO had a potent vasodilating effect. With NO vasomotricity became less evident which might indicate a protective action against vasospasm. Since the neural control was abolished by death, it proves that vasoactive responses to ET-1 and NO are independent of central and autonomic regulation and due to local mechanisms. Time-frequency analysis of intraarterial pressure using the wavelet transform was found to be valuable for quantifying the patterns of intrinsic vasomotricity.

■ 4242

Effects of high dose Triamcinolone Acetonide on proliferation of retinal endothelial cells in a rop-mouse-modelAKKOYUN I (1), YILMAZ G (1), OTO S (1), HABERAL N (2), BACANLID (3),
TUFAN H (4), AKOVA YA (1)

(1) Dept. of Ophthalmology, Baskent University, Faculty of Medicine, Ankara

(2) Dept. of Pathology, Baskent University, Faculty of Medicine, Ankara

(3) Breeding Center, Baskent University, Faculty of Medicine, Ankara

(4) Dept. of Pharmacology, Baskent University, Faculty of Medicine, Ankara

Purpose To report the effects of different triamcinolone acetonide (TA) concentrations on retinal endothelial cell proliferation in a retinopathy of prematurity (ROP) mouse model.

Methods Twenty-four C57BL/6 mice were exposed to 75% oxygen from postnatal day 7 to 12. On day 12, 10 mice were injected 20mg/ml TA intravitreally (IV) while 14 mice were injected 40mg/ml IV TA in one eye (study groups). The contralateral eyes were injected isotonic saline (control group). On day 17, the mice were sacrificed and the eyes were removed for quantitative analysis of preretinal neovascularisation. Neovascularisation was quantified by counting the vascular cell nuclei on the vitreal side of the inner limiting membrane of the retina. For statistical analysis Mann-Whitney U-Test was used.

Results The ROP model revealed a significantly lower neovascular cell count in the study group of 20mg/ml (1.4±1.7 cells per histological section, p<0.00001) and 40mg/ml (0.9±1.4 cells/section, p<0.00001) IV TA compared with the control group (17.0±4.8 cells/section). However there was no significant difference in the cell count between 20 and 40 mg/ml IV TA (p=0.430) injections.

Conclusion These results correlate with previous findings that IV injected high dose TA suppresses endothelial cell proliferation (ECP) in a ROP animal model in vivo. Further studies are necessary to show interactions in suppressing ECP by using intravitreal angiogenic or angiostatic agents for intraocular neovascular diseases.

■ 4243

Retinal toxicity of intravitreal triamcinolone acetonide at high doses in the rabbitMONTERO J (1), RUIZ-MORENO JM (2, 1), BAYON A (3), RUEDA J (4),
VIDAL LOPEZ M (5)

(1) Instituto Oftalmológico de Alicante, VISSUM, Vitreo-Retinal Unit, Alicante

(2) Department of Ophthalmology, Miguel Hernandez University School of Medicine, Alicante

(3) Ophthalmology Service. Veterinary Clinical Hospital, Murcia University School of Veterinary, Murcia

(4) Department of Histology and Anatomy, Miguel Hernandez University School of Medicine., Alicante

(5) Department of Ophthalmology, Murcia University School of Medicine, Murcia

Purpose To study acute retinal toxicity of intravitreal triamcinolone acetonide (TA) at high doses in an animal model

Methods Thirty New Zealand albino rabbits were used. The animals were divided in five groups: Group 1 received an intravitreal injection of 0.1 ml balanced salt solution; Group 2, 0.1ml of the solvent (0.99mg of benzyl alcohol); Group 3, received 4mg/0.1ml TA; Group 4, 20mg/0.1ml TA; and Group 5, 30mg/0.1ml TA. A standard light and dark adapted electroretinogram (ERG) was obtained prior and 30 days after the injection. The eyes examined by electron (EM) and light microscopy (LM) using hematoxylin-eosin, Nissl fluorescent, and immunohistochemistry (glial fibrillary acidic protein).

Results No statistically significant differences in ERG before and 30 days after the injection were found. LM and EM did not show retinal damage in any animal. One eye developed bacterial endophthalmitis 14 days after the injection.

Conclusion Intravitreal TA up to 30mg does not seem to have toxic effects on the function (ERG) or the structure (LM, EM) of the retina of albino rabbits.

■ 4244

Bevacizumab (Avastin) has no toxicity on cultured cell from the anterior and posterior segment of the human eyeWELGE-LUSSEN U, KERN T M, YU AL, KAMPIK A, NELIBAUER AS
Department of Ophthalmology, Ludwig-Maximilians-University, Munich

Purpose For the treatment of age related macular degeneration with choroidal neovascularisation bevacizumab (Avastin) seems to be a promising tool. Since in other cellular systems VEGF has protective effects, the goal of this study was to evaluate the short-term toxicity and safety of varying doses of bevacizumab on cells of the anterior and posterior segment of the human eye.

Methods Primary human retinal pigment epithelium cells (RPE), human optic nerve head astrocytes (ONHA), human trabecular meshwork cells (TMC) and cornea buttons, not suitable for transplantsations were exposed to bevacizumab (25 µg/ml, 250 µg/ml and 2500 µg/ml). for 48 hours. Bevacizumab-related toxicity was evaluated by a colorimetric test (MTT) measuring the inhibition of RPE, ONHA and TMC cell proliferation. Cell viability was also quantified by live-dead assay. Corneal endothelium was measured by phase contrast microscopy.

Results Bevacizumab showed adverse effects on primary RPE cell proliferation and cell viability at a concentration of 2500 µg/ml. Lower concentrations of 25 µg/ml, 250 µg/ml had no influence on RPE cell proliferation and cell viability. There was no effect at all investigated concentrations on human ONHA, TMC and corneal endothelium by all investigated concentrations.

Conclusion In this study blocking of VEGF with a 10 fold concentrations of bevacizumab compared to the common clinical use had a toxic effect to primary RPE. There was no toxicity on other cell types of the anterior and posterior segment. Therefore the clinical use of bevacizumab at a concentration of 1 mg per eye seems to be a safe procedure.

■ 4245 / 448

Adrenergic receptors in retinal arterioles

GISLADOTTIR S, EYSTEINSSON T, SIGURDSSON SB

Physiology, University of Iceland, Reykjavik

Purpose To examined the role of adrenergic receptors, in the smooth muscles of retinal arterioles, in the control of blood flow.

Methods Segments of retinal arterioles from bovine eyes were dissected out and placed in a small vessel myograph. The vessels were continuously bathed with a physiological saline solution (15 ml volume), at a temperature of 37°C and constant oxygen flow. Drugs were added to the bath accordingly (with minimum of n=5) and the contractile or dilative response recorded with the myograph (in mN).

Results The effect of the alpha and beta agonist noradrenaline (norepinephrine) was tested and evoked a significant contractile response. Beta-agonists gave no significant response (isoproterenol, an unspecific beta agonist and terbutalin, a specific beta₂ agonist). An unspecific alpha agonist (dihydroergotamine) and a specific alpha₁ agonist (cirazoline) elicited a significant contractile responses. A specific alpha₂ agonist (clonidine) elicited no significant response. Unspecific beta blockers (propranolol and timolol) significantly relaxed the contraction enhanced by noradrenaline, but only at high doses. The non-specific alpha blocker (phentolamine) significantly relaxed the contraction induced by noradrenaline and cirazoline.

Conclusion These results indicate that in the smooth muscles in bovine retinal arterioles, alpha₁ receptors are dominant. Noradrenaline and cirazoline are mediating their contractile effects through alpha₁ receptors whereas the alpha blocker phentolamine is blocking this response. It is also likely that beta receptors are not present and that the relaxation mediated by the beta blockers might be working through other mechanisms than the blockage of beta receptors, since they affected the noradrenaline response only at high doses.

■ 4246 / 449

Retrobulbar Use of Poloxamer for Ocular Drug Delivery

VEHANEN K (1), HORNOF M (2), URTTIA (2), LIUSITALO H (1)

(1) Department of Ophthalmology, University of Kuopio, Kuopio

(2) Viikki Drug Discovery Technology Center (DDTC), University of Helsinki, Helsinki

Purpose Use of injectable biopolymers for the controlled drug delivery will provide an option between the eye drops and surgically inserted implants. This study was undertaken to demonstrate the suitability of Poloxamer 407 (BASF) as a vehicle of controlled drug release as retrobulbar injections.

Methods Young Wistar rats were anaesthetised before retrobulbar injection of Poloxamer (25 % in 0.9 % NaCl with or without fluorescence markers). Control animals received retrobulbar injections of sodium hyaluronate. The release of fluorescence marker was detected by Fluorotron Master fluorophotometer (Ocumetrics). Rats were euthanised with CO₂ after 6, 12 and 24 hours, 3 and 7 days. Eyes were enucleated, embedded into paraffin and cut into 5 µm sections. Sections were stained for haematoxylin/eosin and immunostained for plasma fibronectin (CCBD), tenascin and inflammation marker (CD38) using 3,3'-diaminobenzidine as a chromogen. The analysis was done using light microscopy.

Results The gelation temperature of the 25 % Poloxamer formulation is 19°C and the gel dissolves in six hours in vitro. In vivo the gel containing FITC-Dextran is visualized parabolically near the site of injection six hours and very faintly 12 hours after the injection. FITC-Dextran was totally disappeared after 24 hours. Clinical evaluation of the eyes and histological analysis do not show pathological changes during the first week after injection.

Conclusion Retrobulbar injection of Poloxamer will give a release of compounds like FITC-Dextran for at least 12 hours. Based on the clinical and histopathological evaluation Poloxamer seem to be avoid of local toxicity. For clinical purposes a more prolonged dissolution time would be desired.

■ 4247 / 450

Structural Aspects of the Design of Ocular Drug Delivery Systems

MAHOMED A, TIGHE BJ

Biomaterials Research Unit, Aston University, Birmingham

Purpose Use of a contact lens as an alternative vehicle to drops offers an opportunity to prolong the contact between the drug and cornea; thus offering a promising route for controlled ocular drug delivery. The potential of current soft lens materials were investigated for this application.

Methods Nelfilcon A, a PVA based lens material, is unusual since it is the only neutral daily disposable lens. This means that drug retention should be primarily dependent on the octanol/water partition coefficient ($\log K_{ow}$), values <0 = higher water solubility, >0 = higher octanol/lipid solubility, rather than on interaction of chemical groups. The $\log K_{ow}$ for a range of materials, drugs and drug models have been calculated and release kinetics from PVA and HEMA/NVP materials compared.

Results The $\log K_{ow}$ of lidocaine, a model ocular anaesthetic, is +2 when the drug is unionised. However drugs tend to ionise at physiological pH, thus the pH dependent octanol/water distribution coefficient ($\log D$) is probably a more valuable parameter. For example $\log D$ for lidocaine increases from -1 to +1.5 as pH increases from 4 to 8. In addition to the influence of $\log D$ to drug delivery, the release kinetics of a drug model from PVA and HEMA/NVP based lenses showed that the latter tended to interact strongly with the drug's acidic groups.

Conclusion The selection of an appropriate hydrogel-drug combination is a critical design aspect for controlled delivery. Macromer technology, e.g. nelfilcon A, which does not require a post-fabrication lens extraction process offers a practical manufacturing advantage. Release profiles are likely to be influenced by both octanol/water partition coefficients and specific chemical interactions. An appropriate "trigger" may be required to accelerate in-eye release.

■ 4251**Techniques to evaluate OBF**

SCHMETTERER L (1, 2)

(1) *Clinical Pharmacology, Vienna*(2) *Biomedical Engineering and Physics, Vienna*

Purpose In the recent years a number of innovative techniques for the assessment of ocular blood flow have been introduced. This presentation gives a short overview of currently available technology.

Methods Noninvasive techniques available for the measurement of ocular hemodynamic parameters include angiography-based systems, devices that use the optical Doppler shift, ultrasound-based systems and devices that assess the pulsatile component of ocular blood flow.

Results For each method the principle, the limitations, the reproducibility and the sensitivity is discussed.

Conclusion Although a number of techniques are available for the measurement of ocular blood flow in humans none is free of limitations. Accordingly, clinical trials should include more than one technique to check the results for consistency.

■ 4252**Retinal oxymetry**

STEFANSSON E (1), HARDARSON SH (1), HALLDORSSON GH (2)

KARLSSON RA (2), BENEDIKTSSON JA (2), EYSTEINSSON T (1, 3), BEACH JM (2)

HARRIS A (4)

(1) *Ophthalmology, Reykjavik*(2) *Electrical and computer engineering, Reykjavik*(3) *Physiology, Reykjavik*(4) *Ophthalmology, Indiana*

Purpose Evaluation of ischemic eye diseases such as diabetic retinopathy, retinal vein occlusions and possibly glaucoma should take into account the metabolic impairment (hypoxia?) in the retina or optic nerve. We have developed an automatic noninvasive spectrophotometric oximeter in order to evaluate oxygen saturation in ischemic eye diseases and its response to treatment.

Methods The automatic oximeter is based on a fundus camera and is just as easy to use. The image is split into 4 monochromatic images and the oxygen saturation calculated from the 586nm and 605nm images. The instrument delivers a pseudocolour map of the fundus, where the oxygen saturation of the arterioles and venules is indicated by colour and numerical values of oxygen saturation and diameter of vessels are supplied.

Results The oximeter is sensitive to breathing of different oxygen mixtures and provides reproducible results. In healthy individuals the oxygen saturation is 96±9% (n=16) in arterioles and 55±14% in venules. This rises to 101±8% and 78±15% (p<0.0001) when breathing 100% oxygen. The venular oxygen saturation is depressed in retinal vein occlusions and this is reversed by scattered laser treatment. Preliminary studies in diabetic retinopathy and glaucoma are under way.

Conclusion The automatic noninvasive spectrophotometric oximeter is easy to use, reliable and sensitive. Preliminary clinical studies indicate that the oximeter can measure hypoxia in ischemic eye diseases and the response to (laser) treatment.

■ 4253**Correlation between Colour doppler and Ocular Pulse Amplitude in glaucoma**

STALMANS I (1), ZEYEN T (1), HARRIS A (2)

(1) *Dept of Ophthalmology, University Hospital Leuven, Leuven*(2) *Dept of Ophthalmology, Indiana University School of Medicine, Indianapolis*

Purpose To evaluate the correlation between retrobulbar blood flow velocities and ocular pulse amplitude (OPA) in healthy subjects and in glaucoma patients.

Methods Three study groups were designed: healthy subjects, patients with normal tension glaucoma and high tension glaucoma. These subjects underwent OPA, as well as flow velocity measurements by means of color doppler imaging (CDI) of the central retinal, nasal and temporal short posterior ciliary arteries and ophthalmic artery. The correlation between these measurements was estimated using a Spearman correlation test.

Results In the healthy subjects, a correlation was found between the OPA and the systole/diastole ratio (S/D) as well as resistive index (RI) of all 4 measured retrobulbar vessels. This correlation was much weaker in both groups of glaucoma patients.

Conclusion A clear correlation was found between OPA and S/D as well as RI in healthy subjects, but not glaucoma patients. The potential mechanisms and clinical consequences will be discussed.

■ 4254**Prostaglandin analogues' effect on ocular blood flow**ORGIL S, ZAWINKA C, KATAMAY R, REINHARD G, FLAMMER J
University Eye Clinic, Basel

Purpose To compare the effect of travoprost 0.004% and latanoprost 0.005% on ocular blood flow in glaucoma patients.

Methods After washout, IOP in both eyes (Goldmann), choroidal blood flow (laser Doppler flowmetry) and retinal vessel diameter (Retinal Vessel Analyzer) in one randomly selected eye were measured at baseline, after two weeks and after 4 weeks of treatment with travoprost or latanoprost QD, in a randomized, double masked 2-way cross-over study in 20 open angle glaucoma patients. Results were analyzed in a two by three ANOVA model.

Results After a 4 week treatment period, latanoprost reduced IOP from (mean ± SD) 19.6 ± 3.5 to 15.2 ± 3.2 mmHg and from 21.0 ± 5.0 to 15.2 ± 3.6 mmHg in the right and the left eyes respectively, while travoprost reduced IOP from 19.1 ± 3.7 to 15.7 ± 2.5 mmHg and from 20.6 ± 4.9 to 15.9 ± 3.7 mmHg in the right and the left eyes respectively. While IOP reduction was significant for both eyes ($p<0.0001$), the effect was comparable with both substance ($p=0.21$ and $p=0.18$ for the right and the left eyes respectively). Perfusion pressure in the experimental eye increased in average by $6.1 \pm 7.2\%$ ($p=0.003$). While choroidal blood flow seemed to remain stable ($p=0.58$), retinal arterioles showed a tendency to constrict by $2.4 \pm 3.5\%$ ($p=0.029$), latanoprost and travoprost showing a comparable effect ($p=0.63$).

Conclusion Prostaglandin analogues may induce a mild retinal arteriolar constriction in some glaucoma patients. Because the expected decrease in blood flow of approximately 9.3% with an arteriolar constriction of 2.4% is very close to the expected increase in blood flow of 6.1% due to the observed increase in ocular perfusion pressure, the observed arteriolar response may simply represent an autoregulatory response.

■ 4255

Carbonic Anhydrase Inhibitor's effects on ocular blood flow

HARRIS A, SIESKY B, MARQUES C, YUNG CW, SINES D

Department of Ophthalmology, Indiana University School of Medicine, Indianapolis

Purpose According to the European Glaucoma Society factors involved in the etiology and progression of glaucomatous optic neuropathy are not only pressure dependent but are related to the vascular supply to the optic nerve head. Epidemiologic studies including the Baltimore, Barbados, Egna-Neumarkt and Proyecto VER eye studies found several vascular risk factors contribute to glaucomatous disease. These include diabetes mellitus, systemic hypertension, migraine, female gender, disc hemorrhage and low diastolic perfusion pressure in different countries and across different ethnic groups. Additionally, non-epidemiologic studies have found numerous glaucomatous ocular hemodynamic deficits including a recent longitudinal study which found low ophthalmic artery blood flow velocity to be a risk factor for glaucoma progression.

Methods Carbonic anhydrase inhibitors (CAI) have been shown to enhance ocular hemodynamics in clinical trials. These medications induce vasodilation utilizing a similar physicochemical cascade to carbon dioxide (CO₂) breathing. Topical CAIs block the hydrolysis of CO₂ to bicarbonate in ocular tissue which also may result in a localized elevated CO₂-induced vasodilation.

Results It is important to note that ocular hemodynamics remain only a surrogate for tissue oxygenation; however, in animal studies topical CAI's have increased retinal oxygen tension. As imaging technologies have improved, similar oxygen assessment can now be performed non-invasively in humans.

Conclusion Both the epidemiologic and non-epidemiologic data indicates a link between glaucoma and hemodynamic deficits that strongly support the need to conduct large scale prospective studies to assess the effects of modulating the ocular blood flow in glaucoma.

■ 4256

Additive effect of topical CAIs to prostaglandins on IOP lowering

HOLLÓ G

Department of Ophthalmology, Semmelweis University, Budapest

Purpose To investigate the additional IOP lowering effect of brinzolamide, a topical carbonic anhydrase inhibitor (CAI) added to topical travoprost in open-angle glaucoma and ocular hypertension, and to review the potential background mechanisms.

Methods In a prospective, double-masked, randomised, active controlled, parallel designed study (the TATS study) patients were treated with travoprost 0.004% QD for 4 weeks, then were randomised to additional timolol 0.5% (n=95) or brinzolamide 0.1% (n=97) BID. IOP was measured at 8.00 a.m., 12.00 p.m. and 18.00 p.m. at baseline (on travoprost only), and at 12 weeks of combined medication.

Results Despite of the known difference in IOP lowering with timolol and brinzolamide in monotherapy, in the TATS study both drugs reduced IOP equally well when added to travoprost. No statistically significant difference was found between the two arms in IOP reduction at any time point or in the mean diurnal IOP (mean diurnal IOP decrease: 3.2±2.4 mm Hg for timolol and 3.4±2.1 mm Hg for brinzolamide, p=0.55). Literary search suggests that travoprost may increase the activity of carbonic anhydrase in the ciliary epithelial cells. This may lead to an excess aqueous humour production, which may be blocked by CAIs, which therefore may be more effective in a combination with prostaglandin analogues than in monotherapy.

Conclusion Topical CAIs may be especially effective in IOP reduction when added to prostaglandin analogues, as shown in the TATS study for brinzolamide in an evidence based manner. The background of this effect requires further laboratory research.

■ 4257

Additive effect of topical CAIs to prostaglandins on ocular blood flow

STALMANS I

Dept of Ophthalmology, University Hospital Leuven, Leuven

Purpose Previous studies have suggested that topical CAIs may be particularly effective when added to prostaglandin analogues. This study was designed to evaluate the effect of brinzolamide on ocular blood flow velocity, when used in addition to travoprost.

Methods Color doppler was used to measure retrobulbar blood flow velocities after washout from current medications (baseline), after 4 weeks of travoprost monotherapy, and after addition of brinzolamide for 3 months.

Results As a short communication, the preliminary results of this study will be presented.

Conclusion As a short communication, the preliminary results of this study will be presented.

■ 4261

When to decide on non-organic visual loss?

SPILEERS W

Dept of Ophthalmology, Leuven

Purpose When the patient tells that he cannot see and the doctor can not immediately see why, a non-organic visual loss has to be excluded.

Methods Different testing methods can be of value in deciding on organic versus non-organic visual loss: visual acuity and visual field measurements, pupillary reflexes, electrophysiologic testing, imaging techniques, ...

Results The value of these different methods will be discussed and illustrated.

Conclusion Deciding on non-organic versus organic visual loss is a complex decision task.

■ 4262

Conditions that may mimic non-organic visual loss

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 conditions that need to be excluded when non-organic visual loss is suspected.

Methods A case presentation format will be used to illustrate those conditions which can be discovered using visual electrophysiological tests in those patients in whom a non-organic origin for visual loss is suspected.

Results Conditions such as Stargardt macular dystrophy, X-linked retinoschisis and cone dystrophy in their early stages, autosomal dominant optic atrophy or Batten disease all need to be excluded when visual loss is thought to be non-organic. ISCEV-standard full-field flash electroretinography, pattern electroretinography and visual evoked potentials contribute significantly to making the correct diagnosis in this instance.

Conclusion Visual loss in a list of organic conditions may mimic non-organic visual loss. Electrophysiology is essential in differentiating true organic from non-organic visual loss.

■ 4263

The role of electrophysiology in medically unexplained visual loss

HOLDER GE

Moorfields Eye Hospital, London

Purpose To discuss the role of electrophysiological examination in the assessment of medically unexplained visual loss.

Methods The test armamentarium incorporates full-field (ERG), pattern (PERG) and multi-focal electroretinography (mfERG), and visual evoked potentials (VEP) to pattern appearance, pattern reversal and diffuse flash stimulation.

Results The diagnostic use of electrophysiological recording will be illustrated in a variety of cases including loss of visual acuity with a normal fundus examination; assessment of the night blind patient with a normal fundus; and non-organic visual loss.

Conclusion The objective data provided by electrophysiological recording are indispensable to the diagnosis and management of this group of patients. In those patients where abnormal function is not accompanied by visible changes in the fundus, electrophysiology enables the characterisation of the disease and may suggest potential targets for mutational screening. In non-organic visual loss the role of electrophysiology is to demonstrate normal function in the presence of symptoms that suggest otherwise.

■ 4311**Advanced imaging and quantitative evaluation techniques of the ocular surface using confocal microscopy**

STACHS O, ZHIVOV A, GUTHOFF RF
Ophthalmology, University of Rostock, Rostock

Purpose Over the past two decades, the applications of in vivo confocal microscopy to the investigation of ocular surface anatomy and diseases in the living eye have been greatly extended. Confocal microscopy enables detailed investigation of tarsal and palpebral conjunctiva, central and peripheral cornea, tear film, and lids, as well as it allows evaluation of the ocular surface at the cellular level. High-quality imaging in both contact and non-contact modes offers a new understanding of the functions of the ocular surface system, and in the coming years, such knowledge will become increasingly comprehensive and specific. Confocal microscopy may provide a link between well established ex vivo histology and in vivo study of ocular pathology, not only in clinical science but also in clinical practice. The purpose of this contribution is to summarize the current knowledge, advanced imaging and quantitative evaluation techniques using in vivo confocal microscopy of the ocular surface.

■ 4312**Microscopic imaging of limbus and conjunctiva**

MASTROPASQUA L
Ophthalmic Clinic - Center of Excellence in Ophthalmology, Chieti Pescara

Purpose Laser scanning in vivo confocal microscopes (IVCM) provide the opportunity to image, the microscopic architecture of the transparent corneal tissues and also of non-transparent structures such as limbal and conjunctival surface. The imaging of the limbus, a highly vascularized, innervated region, which harvests corneal epithelial stem cells (LSC), represent a challenge in ocular surface diagnostic exams. The fact that corneal epithelial stem cells reside outside of the cornea proper suggests that studying the corneal epithelium without taking into account its related limbal tissue will furnish only partial pictures. Ocular surface epithelia show a microscopic pattern of transition moving from conjunctiva towards limbus (with its unique structures such as Vogt's palisades and crypts) and cornea, which needs to be considered when analyzing in vivo limbal epithelium. IVCM provides images of the limbus and related epithelial transition zones which well compare with impression cytology of the limbal area. However LSC, located in protected basal crypts can not be discerned by morphology. IVCM furnishes only morphological analysis with limited phenotypic cell and tissue characterization, but provides fine indications and suggests mechanistic understanding of what occurs in limbal disease affecting LSC function such as chemical burns, infections, chronic limbitis, ulcers and other diseases. The formation of corneal conjunctivalization is often preceded by in vivo identifiable microscopic signs of limbal damage such as inflammation, necrosis and loss of the transition pattern. Moreover IVCM may help ophthalmologists in the evaluation of cellular changes after ocular surface surgery involving the limbus such as limbal and amniotic membrane transplantation.

■ 4313**The pro's and con's of imaging corneal and limbal nerves with in vivo confocal microscopy**

MÜLLER LJ (1), TERVO T (2), KRUISE FE (3)
(1) *Ocular Signal Transduction, Amsterdam*
(2) *Ophthalmology, Helsinki*
(3) *Ophthalmology, Erlangen*

Purpose The non-invasiveness of in vivo confocal microscopy (IVCM) makes it a useful tool to study human corneal nerves. In this presentation the pros and cons of IVCM of corneal and limbal nerves will be explained by comparing in vivo images with the underlying structures in tissue.

Methods Corneal nerves were recorded with a TSCM, Model 165A (Helsinki, Finland) and a HRT II + cornea module (Erlangen, Germany). Fresh corneas from the operating theatre and from the Cornea Bank Amsterdam were either processed for electron microscopy or stained with gold-chloride and antibodies against neuropeptides present in the nerves.

Results With IVCM it is relatively easy to visualize nerves below the basal epithelial cells. These nerves consist of 1-7 straight and 1 beaded nerve fiber(s) in the cornea and 1-3 in the limbus where the beads, studded with mitochondria, reach sizes of 2 µm across. The nerve morphology may change with eye diseases (e.g. keratoconus, diabetes, dry eyes) and with refractive surgery. As IVCM images are ~ 0.4x0.4 mm, the data on orientation and density of the nerves in the cornea appeared to be inconsistent. This inconsistency can now be explained by the V-shaped organization based on elaborated analysis of gold chloride stained tissue and verification by others using the HRT II. This device clearly shows radially arranged nerves along the circumference in the limbus, but not the interwoven stromal nerves as seen with immuno-stainings.

Conclusion Despite fast screening and increased resolution of recent in vivo confocal microscopes the exact location of the recordings is arbitrary. Since nerve terminals and nerve content cannot be investigated, tissue processing is still necessary to verify IVCM observations.

■ 4314**Confocal microscopy in immune and inflammatory responses of the ocular surface**

NUBILE M
Ophthalmic Clinic - University "G. d'Annunzio" Chieti-Pescara, Chieti

Purpose While in vivo confocal microscopy (IVCM) has been widely used in the past years for the imaging of typical corneal resident cells (i.e. epithelial cells, keratocytes and endothelium), both in healthy and diseased corneas, a new diagnostic approach is that related to the evaluation of bone marrow derived cells within the corneal and ocular surface tissues. Globular-shaped cells and dendritic-shaped cells represent distinct morphological cell types clearly distinguishable by using IVCM from the corneal structural cells. However IVCM can not phenotypically characterize these cell subpopulations, providing only micro-morphological analysis, and ongoing investigation are focused on immuno-histochemical comparison, to prove their real nature. Dendritic cells (DCs), a class of professional antigen presenting cells, can be observed in vivo in normal human corneas, at the level of the limbal epithelium, where they are thought to be resident, and it has been shown that in immune-mediated inflammation (i.e. graft rejection, herpes simplex recurrent keratitis, vernal keratoconjunctivitis) an increase in epithelial DCs density at the limbal area together with DCs migration to the central cornea, can be observed by IVCM. Moreover different kind of inflammatory disease of the cornea and conjunctiva are associated with the in vivo evidence of recruitment of globular cells, presumably infiltration of inflammatory cells, within the ocular surface and corneal tissues. The possibility to observe DCs and inflammatory cells, together with the induced tissue damages in such clinical conditions may lead to diagnostic advantages, related to a better comprehension of the inflammatory and immune-mediated changes of the cornea and ocular surface.

= 4315

In vivo confocal microscopy of the conjunctiva and lid margin

MESSMER EM

Dept. of Ophthalmology, Ludwig-Maximilians-University, Munich

ABSTRACT NOT PROVIDED

■ 4321

Oxidative stress markers in diabetic retinopathy

BICHO MP

Genetic Laboratory - Lisbon Faculty of Medicine, Lisbon

Purpose The role of oxidative stress in the pathogeny of chronic diabetic complications in particular retinopathy, is largely documented in the last ten years. Several mechanisms acting at different levels of its natural history are probably implicated. The most ancient now revisited is the increased activity of sorbitol pathway under diabetic or hyperglycemic conditions which includes osmotic stress after accumulation of sorbitol increased cytosolic NADH/NAD ratio, depletion of NADPH and accumulation of fructose with the resulting non enzymatic production of advanced glycation end products (AGES). Consequence of this is the activation of protein kinase C, nitrosative and oxidative stress mediated downstream events such as MAP Kinases and polyADPribose polimerase activation. Oxidative stress is dependent of NADPH oxidase increased activity with production of ROS resulting from the interaction of AGES with its receptors, of protein kinase C and unbalanced activation of defence mechanisms. Also aldose reductase dependent mechanisms are linked to downstream regulation of inflammatory genes. The several cell types of retina are involved namely endothelial cells, pericytes and epithelial pigmentary cells, which can enter in apoptosis process which is accompanied of ischemia/reperfusion lesions not balanced by the several growth factors which can aggravate oxidative stress. Utilization of several blood peripheral markers resulting from these processes are also measured by several authors and us.

■ 4322

Inflammatory markers and blood changes

DE SOUZA RAMALHO P, PEREIRA DA SILVA A

-, Lisbon

Purpose Although the pathogenesis of diabetic retinopathy (DR) is multifactorial there is increasing evidence that components of inflammation seems to be systematically involved in this neurovascular degenerative disease. This evidence comes from animal models and human studies. Within a week of experimental diabetes in the rat, leucocytes accumulate, adhere to retinal blood vessels and migrate through its walls to the neural retina. ICAM-1, VCAM-1 and VEGF increase expression with exudation, oedema and endothelial cell injury (death) leading to capillary occlusion and ischemia (signs of inflammation). Inhibition of leukocyte activity with antibodies or anti-VEGF molecules can prevent early retinal diabetic changes. In non human primates, VEGF induces all the retinal changes of DR including pre-retinal neovascularization. In DR patients, inflammatory molecules and all VEGF isoforms are upregulated in the vitreous. Aspirin have been able to prevent DR in dogs. Less severe retinopathy in rheumatoid arthritis is reported in diabetic patients receiving high doses of aspirin. Retinal oedema and neovascularization in PDR can be reversed with corticosteroids and anti-VEGF therapy. There are few studies in diabetes examining blood markers. Evaluation of adhesion molecules in the blood has produced mixed results. But there are reports of upregulation of ESR, fibrinogen, SDF-1 α , RANTES, EpOx, Haptoglobin and ACE, supporting inflammatory peripheral blood changes, in severe diabetic retinopathy.

■ 4323

Growth factors and pro-angiogenic molecules-survival pathways

PEREIRA DA SILVA A, DE SOUZA-RAMALHO P

-, Lisbon

Purpose Diabetes induces vascular and neuronal sequelae. Anti-inflammatory, antioxidant genetic and angiogenic factors, are involved in diabetic retinopathy (DR). DR involves recruitment of pro-angiogenic molecules through growth factors, leading to cell survival. Hypoxia/ischemia, is a strong triggering factor for VEGF and EPO induction and Transmembrane Reductase (TMR) activation, leading to pro-angiogenic/anti-apoptotic process. TMR is a membrane enzyme, expressed particularly in endothelial cells and erythrocytes, having antioxidant and anti-apoptotic properties. Our studies revealed its down regulation in hypertension, obesity and diabetes, and increase in DR subjects. EPO an haematopoietic cytokine is also pro-angiogenic and neuroprotective, assisting neuronal survival by binding to its receptor in the brain and retina. It is up regulated in proliferative DR. EPO seems related to TMR and Acid Phosphatase of erythrocyte which is involved in the control at membrane level of energy production after activation of glycolysis and response to growth factors like insulin, IGF-1, erythropoietin and others, connected to cell growth and energy metabolism. Recently high concentrations of EPO in the vitreous and aqueous humour in proliferative diabetic retinopathy has been reported. HrEPO administration can provide neuroprotective effects against cell damage in diabetic rats, suggesting that EPO could provide neuroprotection also in diabetic retinopathy. Our preliminary studies indicate a tendency towards higher EPO serum expression in DR. These multifunctional molecules recruited by ischemia/hypoxia-reperfusion, through its pro-angiogenic and anti-apoptotic properties, contribute towards cellular protection in DR.

■ 4324

Genetic factors in diabetic retinopathy

PEGO P (1), BICHO MP (1)

(1) *Genetic Laboratory - Lisbon Faculty of Medicine, Lisbon*

(2) *Department of Ophthalmology - Fernando Fonseca Hospital, Amadora*

Purpose Diabetic retinopathy is a leading cause of adult vision loss and blindness. The duration of diabetes and glycemic control are the two most important factors in the development of retinopathy. However, these factors alone do not explain the occurrence of retinopathy. In fact, some groups of patients develop diabetic retinopathy in a relatively short period of time despite good control, while others may escape retinopathy despite poor control even over a long period. This raises the possibility of a genetic predisposition to retinopathy, which is supported by twin, family and ethnic studies. With the complex metabolic environment of the retina, many risk factors have been proposed. The search for genetic factors in multifactorial diseases is characterised by two approaches: family linkage studies and case control (association) studies. A large number of candidate genes have been examined in patients with diabetes. Some of these candidate genes are genes for aldose reductase, nitric oxide synthase, receptor for advanced glycation end products, angiotensin converting enzyme, human leucocyte antigen, tumour necrosis factor and vascular endothelial growth factor. However few groups have identified a strong association between a gene and the frequency or severity of retinopathy. This presentation will be focus on genes involved in distinct metabolic and functional pathways known to be affected in diabetes and will review the current status of these genes and their association with diabetic retinopathy. We will also reveal the results of our studies in genetic factors in diabetic retinopathy.

■ 4325

VEGF and PEDF, the main regulators of angiogenesis in the retina

MARTINEZ A

Neuroanatomy and Cell Biology, Instituto Cajal, CSIC, Madrid

Purpose Angiogenesis is the formation of new blood vessels from pre-existing ones. This is a common process in normal growth and development, but it is also a main component in the pathophysiology of several diseases. Angiogenesis is regulated by the equilibrium between pro-angiogenic and anti-angiogenic factors. Several diseases of the eye are characterized by an excessive formation of blood vessels and are also related to hypoxic conditions. These include retinopathy of prematurity (ROP), age-related macular degeneration, diabetic retinopathy, and neovascular glaucoma, among others. A basic understanding of the underlying angiogenic mechanism in these conditions may help in devising novel therapies.

Methods We used a variety of cell biology and biochemical methods to study the angiogenic mechanism of VEGF and PEDF.

Results Of all the potential pro-angiogenic molecules, vascular endothelium growth factor (VEGF) seems to be the main promoter of blood vessel growth in the eye. On the other side of the equation we find pigment epithelium-derived factor (PEDF), a 50-kDa glycoprotein highly expressed in the retinal pigment epithelium and which is the principal antiangiogenic and neurotrophic protein of the eye. Interestingly, under hypoxic conditions, VEGF increases through a HIF-1-dependent mechanism whereas PEDF protein decreases (but there is no change in PEDF mRNA levels). Careful biochemical studies have shown that the reduction in PEDF is mediated by VEGF, through the activation of metalloproteinase (MMP)-2 and MMP-9 expression and activity.

Conclusion In summary, drugs able to either reduce HIF-1 activation, decrease VEGF expression, or increase PEDF presence in the eye, may be useful in preventing excessive ocular angiogenesis.

■ 4331

The use of hydroxyapatite implant in enucleations : retrospective review of 689 cases

DESIARDINS L (1), LUMBROSO-LEROUIL L (2), LEVY GABRIEL C (1),

PLANCHER C (3), ASSELAIN B (3)

(1) Ophthalmology, Paris

(2) Ophthalmology, Paris

(3) Biostatistics, Paris

Purpose To study the result of the use of hydroxyapatite orbital implant during enucleation for uveal melanoma or for retinoblastoma**Methods** Retrospective review of patients treated between June 1991 and December 2005. Information concerning the type and size of implant, type of wrapping and post-operative course was registered and a statistical analysis was performed.**Results** 689 hydroxyapatite implants were used during this period; there were 341 adults and 348 children; Scleral wrapping was used for 33 patients, then PTFE for 70 and then vicryl meshwork. The median follow up is 42 months. Complications occurred in 10% of patients with a slight predominance in children ($p < 0.0325$). Most complications consisted in limited implant exposure with spontaneous healing but a few patients presented large implant exposure with chronic conjunctivitis. Late inflammatory complications were observed only with PTFE. The use of PTFE was associated with a higher rate of complications ($p < 0.00297$). Only 3% of patients needed an additional surgical procedure.**Conclusion** Hydroxyapatite implants give satisfactory results in 97% of patients. Vicryl meshwork wrapping can be used but PTFE should be avoided.

■ 4332

Intravitreal methotrexate for managing vitreoretinal involvement of primary central nervous system lymphoma: Nine years of experience.

FRENKEL S (1), SIEGAL T (2), PE'ER J (1)

(1) Ophthalmology, Hadassah-Hebrew University Medical Center, Jerusalem

(2) Oncology, Hadassah-Hebrew University Medical Center, Jerusalem

Purpose To describe our experience in treating vitreoretinal involvement of primary central nervous system (CNS) lymphoma, using intravitreal injections of methotrexate (MTX).**Methods** Patients with suspected intraocular lymphoma underwent a diagnostic vitrectomy. Samples were sent for cytology and genetic evaluation. Treatment protocol: 400 µg/0.1 cc are injected intravitreally under local anesthesia. Injections are administered twice weekly for 4 weeks, once weekly for 8 weeks, and then once monthly for 9 months, for a total of 25 injections. Data were collected from the patients' records.**Results** In the past 9 years we have treated 40 eyes of 23 patients; 6 patients had monocular involvement, and 17 binocular. Twelve patients were initially diagnosed with non-responsive uveitis, and 11 with either CNS or systemic lymphoma with a later involvement of the eye. Four patients had systemic lymphoma and were found to have CNS lymphoma after the ocular involvement. Three patients had T-cell lymphoma, and the rest had B-cell lymphoma. All patients had a complete response after a maximum of 12 injections of MTX. None of the patients had an intraocular recurrence. Among the side effects, the most common was corneal epitheliopathy, which usually appears after the third injection and begins to subside when the intervals between injections increase.**Conclusion** Intraocular involvement of lymphoma can be controlled efficiently and without serious adverse reactions by intravitreal MTX injections. The treatment protocol described herein has so far eliminated the intraocular recurrence. The accumulating clinical data of the treatment results bring us to propose this as a standard treatment guideline.

■ 4333

Orbital involvement of retinoblastoma

HADJISTILIANOU T (1), DE FRANCESCO S (2), ACQUAVIVA A (3),

MENICACCI F (4), GALLUZZI P (5)

(1) Ophthalmology - Ocular Oncology Unit, Siena

(2) Ophthalmology- Ocular Oncology Unit, Siena

(3) Pediatrics, Siena

(4) Ophthalmology, Siena

(5) Neuroradiology Unit, Siena

Purpose To report the therapeutic approaches in an attempt to improve the outcome of advanced cases of orbital involvement of retinoblastoma.**Methods** Between 1980 and 2005, 330 cases of retinoblastoma were treated at the Retinoblastoma Referral Center of the University of Siena. 5 patients out of 330 were treated for orbital involvement which was isolated in 2 patients and associated with metastases in 3 patients (3 within the CNS and 2 out of 3, outside). Treatment included surgery, chemotherapy and external radiotherapy.**Results** 2 patients with isolated orbital involvement are alive and disease free respectively 15 and 16 years after treatment. 1 patient out of 3 with systemic metastases is alive and disease free 2 years after treatment. 1 patient died after 7 months. 1 patient is still under treatment.**Conclusion** Highly aggressive treatment by combining surgery, chemotherapy and external beam radiation therapy allowed complete remission of disease in patients with isolated orbital involvement. Orbital involvement associated with metastases carries a bad prognosis and intensive chemotherapy and radiotherapy may allow a longer survival and in some cases obtain a complete remission.

■ 4334

Punch biopsy for iris lesions: a novel technique for obtaining histology samples

PE'ER J, BLUMENTHAL EZ, FRENKEL S

Ophthalmology, Hadassah-Hebrew University Medical Center, Jerusalem

Purpose To describe a novel technique for obtaining iris biopsy samples for histopathological analysis.**Methods** Through a clear corneal incision with a 20G microvitrectomy (MVR) blade, a "trabeculectomy punch" (Kelly Descemet's Membrane Punch, Katena, USA) with 1.0 mm diameter head and 0.75 mm deep bite was inserted into the viscoelastic-filled anterior chamber. The Kelly Punch was placed over the lesion and pressed down before the punch was made. After taking the sample, the Kelly Punch was removed from the eye and then opened over a dry cellulose sponge. Tissue samples were placed in 4% formalin for standard staining with H&E, PAS and immunostains.**Results** In two iris lesions in which the diagnosis was uncertain, using the punch biopsy technique with the Kelly Punch we were able to obtain a piece of tissue large enough for any histological analysis. H&E staining showed melanoma of spindle cell type. Tissue sections stained positive with MART-1, and negative with Creatine Kinase (CK), establishing the diagnosis of melanoma of the iris.**Conclusion** Iris biopsy with the punch technique yields a tissue sample, as opposed to cytology samples from fine needle aspiration biopsy. The technique is quick, simple to perform, minimally invasive and requires non-expensive and easily available equipment. The tissue obtained is of a quality and quantity enabling routine and special stainings. This approach can be used for iris lesions also in phakic patients.

■ 4335

Utility of Biopsy in cases of Pigmented Iris Tumour

SCHALENBOURG A, UFFER S, ZOGRAFOS L

Jules Gonin University Eye Hospital, Lausanne

Purpose In the presence of pigmented iris lesions evocative of malignant melanoma and implying invasive therapeutic measures, a foregoing biopsy to exclude a benign lesion may seem a reasonable approach. Examining our patient files, we wanted to explore the reliability of such diagnostic procedure.

Methods Retrospective, consecutive histopathologic case series of 10 specimens excised since 1993 in cases of pigmented iris tumor. Histopathologic diagnosis was confronted with clinical diagnosis.

Results Four specimens confirmed clinical suspicion of iris melanoma (40%). Two biopsies contained insufficient material (20%). One enucleation specimen of diffuse iris melanoma showed large zones of benign melanocytic cells (10%). Three biopsies had only nevus cells, whereas clinical data or ulterior histopathologic examinations were compatible with diagnosis of malignant melanoma (30%).

Conclusion In our case series, the risk of an iris biopsy providing an inconclusive or false negative result was 60%. In cases clinically evocative of iris melanoma, utility of a biopsy is debatable.

■ 4336

Intraocular biopsy forceps - A new promising approach

AKGUEL H, JURKLIES B, BORNFELD N

Department of Ophthalmology, Essen

Purpose Generally biopsy is indicated for diagnostic uncertainty of intraocular processes without showing characteristic clinical findings and diagnostic confirmation before treatment. There is an increasing interest for further genetic and immunocytochemical information in order to characterize the neoplasm, especially grading and prognosis of getting micrometastasis in case of uveal melanoma. We have developed a new surgical technique using a special intraocular biopsy forceps.

Methods Seven patients with uncertain intraocular mass got biopsies performed by the special biopsy forceps. Two of seven were biopsies from iris, five of seven from choroid. The pathway for iris biopsy was as usual through a paracentese in local anesthesia, choroideal biopsies were performed under general anesthesia through a standard three-port vitrectomy. For further processing the specimen were submitted immediately to the cytopathologists after flushing out in isotonic saline solution.

Results All biopsies were successful in getting enough specimen for histologic examination. There were no intra- and postoperative complications.

Conclusion Biopsy by the special forceps is a new promising approach. Especially for solid tumors this technique has an advance in getting enough specimen with a united cell structure instead of a cell suspension for improving the histological examination and lowest risk for complications.

■ 4337 / 479

Optico-reconstructive operations in block excision of iridociliar tumours

SAAKYAN SV (1), CHENTSOVA EV (2)

(1) Ophthalmoncology and Radiology, Moscow

(2) Eye Trauma, Moscow

Purpose The development of iridociliar tumours is often followed by complications which worsen the functional outcomes after the tumour excision. Cataract is one of these complications. The aim was to determine the indications and contraindications to simultaneous optico-reconstructive operations in block excisions.

Methods 571 patients with local iridociliar tumour excision were analyzed. Reconstructive operations were performed in 262 patients. Block excision with the simultaneous cataract extraction was performed in 32 patients (5,6%). During the last two years the lens removal has been followed by intraocular correction in 8 patients.

Results The simultaneous tumour excisions and reconstructive operations have let us preserve the high visual acuity in 65,7% of cases in a mean follow-up period of 10,5 years (from 6 months to 20 years). Iridoplasty has been performed in the tumours of size less than 1/3 of the iris. In all cases the eyes were preserved not only as cosmetic but as functioning organs. The by-layer and through sclerokeratoplasty has been performed in the iridociliar tumours, invading sclera and limb. Intraocular correction was performed in iris with defect of less than 1/3 of its area and in this case the first step was tumour excision, then lens removal, iridoplasty and afterwards intraocular lens was inserted and fixed to the sutured iris.

Conclusion Thus the main contraindication for the block excision done simultaneously with intraocular correction are the excised tumour's volume and area of iris defect. The combined method of tumour excision and simultaneous cataract extraction plus intraocular lens insertion has allowed to acquire high visual acuity in 72% of patients and to preserve eye form and size in all the cases.

■ 4341

Software for the semi-automatic determination of the retinal vessels diameter

MONTEIRO-GRILLO M (1), LOPES D (1), MORA A (2), FERREIRA A (1),
FONSECA J (2), VIEIRA P (2)

(1) Clínica Universitária de Oftalmologia - Hospital de Santa Maria, Lisbon

(2) Faculdade de Ciências e Tecnologia - Universidade Nova de Lisboa, Lisbon

Purpose A low retinal arteriolar to venular diameter ratio (A/V ratio) has been suggested to reflect generalized vascular alterations and to predict the risk of cardiovascular diseases. The purpose of this study was to develop a software for the semi-automatic determination of the retinal vessels caliber, with the final goal of obtaining a standardization of the normal A/V ratio.

Methods We have done a preliminary study with 25 healthy patients (50 eyes), with an mean age of 40.16 years. The software uses digital image processing techniques for detecting and measuring vessels diameter. Non-mydriatic retinal photographs are used. The first step is to measure the optic disc diameter in pixels to compute the ratio between pixels and micrometers, considering a reference optic disc diameter of 1850 µm. In the next step, the user selects the vessels that are analyzed on a square section of 300 µm. The analysis is based on an image segmentation to separate the vessel from the background and to measure the mean diameter using the vessel area and length. The diameter of five arterioles and five venules is measured in a predetermined area, and the A/V ratio is automatically shown.

Results The analysis of these 50 patients showed an average A/V ratio of 0.75, a 0.13 standard deviation, with a high reproducibility.

Conclusion We believe this is a good method for detecting, measuring and obtaining an AV ratio and therefore this relationship can be a good indication to evaluate the retinal vessels.

■ 4342

Age-dependence of dynamic retinal arterial reaction to monochromatic flickering light in a representative sample of normal volunteers

KOTLIAR KE (1), BOCK A (2), SCHMIDT-TRUCKSAS A (2), HALLEM (2),
VILSER W (3), LANZL I (1)

(1) Department of Ophthalmology, Munich University of Technology, Munich

(2) Department of Preventive Sports Medicine, Munich University of Technology, Munich

(3) Biomedical Engineering, Ilmenau University of Technology, Ilmenau

Purpose Human retinal vessels and their reaction to stimuli change during life due to physiological, genetic and pathological influences. Using the Dynamic Vessel Analyzer (DVA) it is possible to assess changes in retinal vessel diameters in response to vasoactive stimuli.

Methods Retinal arterial vessel reaction to flicker light was assessed with DVA in the right eye of 155 medically healthy volunteers. The volunteers were divided in 3 age groups: young (20-37 years), middle age (38-52 years) and seniors (53-70 years). Monochromatic rectangular luminance flicker stimulation (530-600 nm, 12.5 Hz, 20 s) was applied. Data analysis was performed independent from DVA program.

Results In most volunteers a quick retinal arterial dilation in comparison to the baseline as well as an ensuing reactive arterial constriction were observed. In detail we found arterial reactions in % to baseline:

..... young middle age seniors

mean vessel dilation

at the end of stimulation ... 3.2 ± 2.1% ... 3.9 ± 2.2% ... 2.3 ± 2.3

mean maximal constriction

following the stimulation ... 2.6 ± 1.8% ... 1.7 ± 1.5% ... 1.3 ± 1.4

We found statistically significant differences in the parameters between two youngest groups and the oldest age group as marked with * (p<0.05) and ** (p<0.01), U-test with Bonferroni correction.

Conclusion Even a „healthy older person“ shows an altered vessel reaction compared to a healthy young person. Application of flicker stimulus to retinal vessels may represent a possible method to assess the health state of vessels of central circulation.

■ 4343

Response of retinal vessel diameters to flicker-light in vasospastics

ZAWINKA C, RICKENBACHER I, REINHARD G, FLAMMER J, ORGUL S
University Eye Clinic, Basel

Purpose The aim of the present study was to compare the flicker-light induced change in retinal vessel diameters in vasospastics and age and sex matched normals.

Methods 15 vasospastics and 15 non-vasospastics were included in this observer-masked, controlled, parallel group study. Subjects were classified as vasospastics if they related a clear history of frequent cold hands, which was quantified by nailfold capillaroscopy. Retinal vessel diameters were investigated using the Dynamic Vessel Analyzer® (Imedos, Jena, Germany). Venous and arterial diameters were measured at baseline and during 30 sec of flickering light provocation which was produced by an optoelectronic shutter. This procedure was repeated three times with 50 seconds of continuous light in between the flicker periods. Diameter changes were calculated as percent changes from baseline and the last 20 seconds of the flicker stimulation. The mean of the three values of both the artery and the vein were calculated.

Results Arteries and veins dilated in the order of 4% in the non-vasospastic group. Our results indicate that the vasospastic group had a significant reduced response to luminance flicker light in the arteries compared to normals (ANOVA, p<0.02), whereas the venous dilation was comparable in both study groups (ANOVA, p=0.4).

Conclusion In vasospastics the regulatory capacity seems to be affected in the retinal vessels. Our data give further evidence that primary vasospasm is also affecting the eye. To elucidate the mechanism and importance for eye diseases further studies are required.

■ 4344

Retinal Blood Flow and Nerve Fiber Layer Measurements in Normal Tension Glaucoma

BERISHA F (1, 2), FEKE GT (1, 2), PASQUALE LR (3, 2), HIROSE T (1, 2),
MCMEEL JW (1, 2)

(1) Schepps Retina Associates Foundation, Boston

(2) Ophthalmology, Harvard Medical School, Boston

(3) Massachusetts Eye & Ear Infirmary, Boston

Purpose An unexpected inverse correlation between optic nerve head capillary blood speed and retinal nerve fiber layer (RNFL) thickness in patients with untreated ocular hypertension was previously reported (Feke et al. BJO 1995;79:1088-92). In this study we investigated the relationship between retinal arterial blood flow and RNFL thickness in normal tension glaucoma (NTG).

Methods Eleven patients with early to moderate NTG (age 60.6±10.3 years) and 11 age-matched healthy controls (age 62.0±7.7 years) were included in the study. Blood column diameter, centerline blood speed, and retinal blood flow rate were measured using the Canon laser Doppler blood flow instrument. The inferior temporal artery was chosen for blood flow measurements in NTG patients. Peripapillary RNFL thickness and optic nerve head parameters were measured using Stratus optical coherence tomography.

Results There were no significant differences in retinal hemodynamic parameters between NTG and controls. NTG patients showed significantly increased cup-to-disc ratios compared to controls (p<0.001). Average RNFL thickness was lower in the NTG compared to the control group (p<0.01). When analyzed in quadrants, superior and inferior RNFL thickness in NTG was significantly reduced compared to controls (p<0.01 each). A significant inverse correlation was found between the retinal blood flow, measured in the inferior temporal artery, and the inferior RNFL thickness in NTG group ($R^2=0.59$, p<0.01).

Conclusion The results showed that within the NTG group a thinner RNFL was associated with a higher retinal blood flow. This phenomenon might be related to the upregulation of the various isoforms of nitric oxide synthase known to occur in glaucomatous optic neuropathy.

■ 4345 / 451

Spatial properties and shortterm vasomotion of retinal vessels in vasospastic subjects

KOCHKOROVA A, GUGLETA K, ZAWINKA C, KATAMAYR R, FLAMMER J, ORGILS S
Ophthalmology, Basel

Purpose The purpose was to analyze spatial and temporal variations of retinal vessels in vasospastic subjects.

Methods Twenty-six women were divided in two equal groups, vasospastics and normal controls. Coefficient of variation (CV) of the vessel diameter was a measure of spatial irregularity. Coefficients of correlation (CC) of successive means and standard deviations of the vessel diameter within a defined time period was the measure of vessel diameter uniformity during vasomotion.

Results CV in arterioles: $8.8 \pm 2.8\%$ and $6.1 \pm 1.7\%$, CV in venules: $3.8 \pm 1.4\%$ and $3.6 \pm 0.9\%$ in vasospastics and normal controls, respectively (planned comparison: difference between groups in arterioles $p=0.007$). CC in controls: 0.11 ± 0.23 and 0.09 ± 0.23 , CC in vasospastics: 0.25 ± 0.40 and 0.24 ± 0.22 , in arterioles and venules respectively (difference between groups as one factor $p=0.038$, and difference between arterioles and venules as the other factor $p=0.77$, interaction $p=0.96$).

Conclusion Retinal arterioles in vasospastic subjects show higher spatial irregularity than normal controls. Both arterioles and venules demonstrate an altered vasomotion pattern in vasospastic subjects.

■ 4346 / 452

The effects of voluntary fasting on retinal and peripheral vessel reactivity – a case study

HEITMAR R, GHERGHEL D
School of Life & Health Sciences, Dept. Vision Sciences, Birmingham

Purpose To study the retinal and peripheral vessels reactivity, as well as the systemic blood pressure (BP) response to repeated voluntary fasting in a young and healthy subject.

Methods Measurements of the retinal vessels (one arteriole and one venule) reactivity to flicker (by means of the Retinal Vessel Analyser-RVA; IMEDOS, Jena, Germany) were undertaken in one healthy male subject aged 21 years after a 20-h period of voluntary fasting and then one hour after a meal; this experiment has been repeated on three separate occasions at the same time of the day. The RVA measurements consisted of 50 seconds baseline followed by three periods of 20 seconds flickering light provocation, each of them followed by 80 seconds observation. A mean value of the three separate measurements was calculated as the final dynamic vessel response. Systemic BP, and peripheral blood flow (by means of capillary microscopy using the CAM1 Laser Doppler Capillary Anemometer, KK Technology, Devon, England) measurements were also performed on the second and third occasion.

Results The retinal vessels reactivity response was significantly lower in both the arteriole (mean dilation = $-0.17 \pm 0.55\%$) and venule (mean dilation = $+4.5 \pm 2.98\%$) under the fasting conditions comparing to that measured one hour after a meal (mean arteriole dilation = $+3.63 \pm 1.00\%$; and mean venule dilation = $+5.13 \pm 0.75\%$ respectively). These differences were statistically significant ($p<0.05$). There were no differences in either BP or capillary microscopy measurements between the two categories of conditions ($p>0.05$).

Conclusion The abnormal vascular reactivity induced by fasting was significant; however, the mechanism and implications of this observation need further investigation.

■ 4347 / 453

Optic Nerve Blood Flow and Retinal Diameter Responses to Flicker Stimulation are Described by a 2nd Order Linear System Model

RIVA CE (1), BONAIUTI M (2), ROVATI L (2)
 (1) *Oftalmologia, Università di Bologna, Bologna*
 (2) *Dipartimento di Ingegneria dell' Informazione, Università di Modena e Reggio Emilia, Modena*

Purpose Optic nerve blood flow (F) and retinal vessel diameter (D) increase in response to increased neural activity. We applied a control system analysis to describe the main dynamic features of this regulatory process.

Methods F was measured by LDF and D by RVA in healthy subjects (age 25–62 years). Neural activity was evoked by diffuse luminance flicker (various frequencies and 1–8 min duration). The F and D time-courses underwent a 10-point lagging running-averaging process and then were fitted with a modified 2nd-order control system G(s) consisting of the cascade of a Proportional-Derivative (PD) term, a 2nd-order Filter (F2) and an Integrator term (INT). The following characteristic parameters of G(s) were determined: the undamped natural frequency (ω) of F2, the damping factor (ξ) of F2, the gain (K) of PD, the rate time (Tv) of PD and the integrator time constant (τ) of INT.

Results The flicker-induced increase in F during stimulation and the decrease in D after cessation of the stimulus could be well fitted by the response of G(s) to the Heaviside step function (correlation $R^2=0.66$). Thus for the F-response, the following results were obtained: $\omega = 2.31 \pm 1.57$ (se) rad/sec, $\xi = 0.28 \pm 0.1$, $K = 180.75 \pm 11.76$, $Tv = 2.67 \pm 1.62$ sec, $\tau = 5.95 \pm 1.93$ sec. For the D-response the following values were obtained: $\omega = 0.14$ (se) rad/sec, $\xi = 0.071$, $K = 0.01$, $Tv = 29934$ sec, $\tau = 38.7$ sec (correlation factor $R^2 = 0.98$).

Conclusion The response of optic nerve blood flow and retinal vessel diameter to diffuse luminance flicker can be described by a second order linear system. The physiological basis of the various terms of the control cascade remains to be established.

■ 4348

Apoptosis and neurite retraction explain the structural and functional loss in the rat model of postnatal hyperoxia

LACHAPELLE P (1), DORFMAN A (2), PINILLA I (3), CUENCA N (4), DEMBINSKA O (1), IOLY S (5), CHEMTOB S (2), CASANOVA C (6)
 (1) *Ophthalmology- McGill University/Montréal Children's Hospital, Montréal, Québec*
 (2) *Pharmacology and Therapeutics McGill University, Montréal, Québec*
 (3) *Department of Ophthalmology, Hospital Universitario Miguel Servet, Zaragoza*
 (4) *Biotecnología, Universidad de Alicante, Alicante*
 (5) *Dép. de biologie, Université de Montréal, Montréal, Québec*
 (6) *School of Optometry, Université de Montréal, Montréal, Québec*

Purpose Postnatal exposure to hyperoxia appears to stop the normal maturation of the retinal function to what is observed at P17 in normal rats. We investigated the mechanisms at the origin of this loss.

Methods Sprague Dawley (SD) and Long Evans (LE) rats were exposed to hyperoxia (n=24 control, 42 exposed) from birth to P6 or P14 following which the retinal structure (histology) and function (ERG) were evaluated at selected time intervals.

Results Synaptophysin (presynaptic marker) confirms the lack of functional synaptic terminals in the OPL of SD and in the OPL and IPL of LE rats. The latter results from a reduction of ON-bipolar cell terminals as seen with mGluR6 staining, as well as with PKC (rod bipolar) and recoverin (cone bipolar) stainings. Similarly, Calbindin staining also revealed a reduction in the number of horizontal cells while bassoon staining (in rod and cone synaptic ribbon terminals) also suggests limited communication from the photoreceptors to the inner retina. Anomalies were significantly more severe in LE rats. Finally, significant ($p<0.05$) TUNEL positive profiles were located in the INL and ONL of LE retina and only in the INL of SD rats.

Conclusion Our results suggest that the thinning of the OPL results from cell death (from apoptosis) and synaptic retraction (mainly cone and rod bipolar cells and horizontal cells) initiated at the level of the inner retina and progressing towards the outer retina (when SD and LE rats are compared). Our findings propose a previously undescribed mechanism of cell death and synaptic retraction that would explain the functional consequences oxygen induced retinopathy. Funded by CIHR and Réseau Vision

■ 4351

The importance of circadian IOP variation, endurance of glaucoma medications, and compliance in the successful treatment of glaucoma and ocular hypertension

ROBIN AL

Ophthalmology and International Health Johns Hopkins University, Baltimore

Purpose To provide an overview of diurnal variation, importance of endurance relating to glaucoma medications and patient compliance.

Methods A survey was conducted of recent published literature on circadian IOP fluctuation, as well as endurance and compliance as they pertain to glaucoma medications. In addition, original work using two different MEMS devices in glaucoma patients will be presented.

Results Recent evidence has suggested a link between circadian IOP fluctuations and an increased risk of glaucomatous damage. IOP measurements conducted during office hours may not be a good predictor of elevated IOP levels that occur in the supine position during the nocturnal period. One important factor that contributes to large diurnal fluctuations in IOP is low patient compliance in taking their glaucoma medications. This underscores the need for medications with enduring efficacy in lowering IOP. Travoprost 0.004% has been shown to provide significant IOP reductions at the end of the day. Simplifying treatment regimens also contributes to patient compliance; therefore fixed combinations may be useful in glaucoma management. Devices measuring patient compliance are presently under development that will assist clinicians in optimizing treatment and educating patients.

Conclusion Fixed combinations, patient education, enduring treatments and eventually compliance monitoring devices, will help us address the issue of compliance in the management of glaucoma.

■ 4352

Fixed Combination clinical results

TOPOUZIS F

Aristotle University of Thessaloniki Ophtalmology II, Papageorgiou Hospital Periferiaki odos Thessalonikis, Thessaloniki

Purpose To provide a review of the fixed combination glaucoma medications

Methods A survey of the current literature on fixed combinations glaucoma products was conducted.

Results Fixed combination agents, such as Duotrav™ (travoprost 0.004%/timolol 0.5%), provide greater IOP lowering efficacy than either of their individual components alone. These medications also have been compared to concomitant therapy with their two individual components regarding their ability in reducing IOP. Specific clinical trial results will be discussed for each fixed combination agent. Fixed combination products also retain favorable safety and tolerability profiles. Once daily dosed fixed combinations should positively impact patient compliance in the chronic treatment of glaucoma and lead to better medical outcomes.

Conclusion Fixed combination glaucoma agents provide greater IOP lowering efficacy than their individual components with the convenience of once-daily dosing.

■ 4353

Associated compliance benefits with fixed combinations

DENIS P

Dpt of Ophthalmology- Hôpital Edouard Herriot, Lyon

Purpose To provide an overview of fixed combination glaucoma medications and their effect on patient compliance.

Methods A literature survey was conducted on fixed combination glaucoma agents.

Results Several benefits of fixed combinations, such as DuoTrav™, were found. Combining agents with different mechanisms of action can provide enhanced IOP lowering efficacy. A single drop eliminates the washout effect that is often observed when the patient does not allow for an adequate amount of time between instillations of their drops. The combination drop also provides a simplified regimen which can lead to improved compliance. The exposure to preservatives in the formulation is minimized by combining the agents into a single drop. This may lead to a lower incidence of side effects, thus improving compliance.

Conclusion Fixed combination glaucoma agents provide many patient benefits that should be expected to improve their compliance in taking their medications and ultimately lead to enhanced management of elevated IOP.

■ 4361

Driver's vision and in-Vehicle information systems: a review

GALE A

Applied Vision Research Centre, Loughborough University, Loughborough

ABSTRACT NOT PROVIDED

■ 4362

Training, bioptic telescopes and practical fitness to drive with reduced visual acuity

KOOIJMAN AC (1, 2), MELIS-DANKERS BJM (2), BUSSCHER RB (3),

BREDEWOUD RA, VAN DELDEN (2), BROUWER WH (3), WITVLIET J (2)

(1) *Laboratory of Experimental Ophthalmology, University of Groningen, UMCG, Groningen*

(2) *Royal Visio, National Foundation for the visually Impaired and Blind, Huizen*

(3) *Department of Neuropsychology, University of Groningen, UMCG, Groningen*

ABSTRACT NOT PROVIDED

■ 4363

Usefulness and shortcomings of standard perimetry for assessment of driving abilities

PETZOLD A (1), RAUSCHER FG (2), BARBLIR JL (2), PLANT G (1)

(1) *Institute of Neurology, University College, London*

(2) *Applied Vision Research Centre, City University, London*

Purpose Visual field defects can affect driving performance and safety. There is a need to assess the level of visual field loss that can no longer be considered safe for normal driving. There are a number of routine screening tests to decide on the presence or absence of visual field defects. Some of the pitfalls using these tests will be discussed.

Methods Visual field loss can result from a large number of diseases, ranging from hereditary conditions and immunological phenomena to vascular events. Many of these conditions have a high probability of causing central visual field defects. In a number of cases both eyes are affected and any resulting binocular scotoma may be relevant to driving. In addition, the general level of residual visual function is also affected, at least in some conditions. This study has investigated whether the processing of different stimulus attributes (colour, contrast, motion) remains normal in what can be classified as "perimetrically" normal area of the visual field adjacent to the scotoma.

Results We will present a number of representative cases drawn from a cohort of 62 patients. The results show many patients who develop frequently binocular scotomata with a remarkable impairment of the visual function in the adjacent visual field. This was particularly so in glaucoma (n=23). In contrast, cases with cortical pathology (n=21) suffer from discrete scotomata with relatively preserved function elsewhere.

Conclusion These findings show that residual visual function in and around the scotoma can vary significantly both across patients and even within the same patient. In other patients, similar selective loss of visual function can also be demonstrated even when the visual field is perimetrically normal. The implication of these findings in relation to driving safety remain to be established. Any assessment based solely on a categorical approach may overlook the implications of the various pathological substrates for visual function in the remaining visual field.

■ 4364

UFOV test performance in patients with central scotomata

DUNNE MCM (1), DAVIES LN (1), RAUSCHER FG (2), EDGAR DF (2),

CHISHOLM CM (2), BARBLIR JL (2)

(1) *Ophthalmic Research Group, School of Life and Health Sciences, Aston University, Birmingham*

(2) *Applied Vision Research Centre, City University, London*

Purpose Previous research has indicated that the Useful Field of View (UFOV), a visual attention test, is able to identify drivers at risk of crashes. The purpose of this study was to compare UFOV test performance in patients with binocular central scotomata to an age matched control group.

Methods The control group included 100 drivers, all of whom were fit to drive according to the UK driving standards. The 54 patients that took part in this study exhibited binocular central scotomata caused either by retinal or cortical damage. Each participant sat the UFOV test once and had a perimetric assessment. Although learning effects were known to arise from repeated UFOV tests, single readings were considered to represent natural or untrained visual attention capability. Total UFOV losses were recorded and peripheral target localisation errors analysed.

Results The control group exhibited an age-related increase in total UFOV losses. Percentiles were thus calculated for six age bands (20-29, 30-39, 40-49, 50-59, 60-69 and 70+). Forty six (85%) of the patients had total UFOV losses that fell within the 95th percentiles calculated for their respective age band. Almost half of the patients exhibited maximum UFOV peripheral target localisation errors in the weakest hemifield (24 patients) or quadrant (19 patients) determined using a binocular high sampling density perimeter developed at City University.

Conclusion These findings suggest that most of the patients examined had a low risk of driving crashes despite experiencing difficulties localising objects in the region of their scotomata. Further investigation should determine whether the UFOV test, with its gross high contrast targets, is sensitive enough to assess driving safety in this patient group.

■ 4365

Loss of visual field sensitivity in relation to driving performance as assessed by HPT and UFOV

RAUSCHER FG (1), BARBLIR JL (1), EDGAR DF (1), CHISHOLM CM (1),

PETZOLD A (2), PLANT G (2), DUNNE MCM (3), UNDERWOOD GJ (4)

(1) Applied Vision Research Centre, City University, London

(2) Institute of Neurology, University College, London

(3) Neurosciences Research Institute, School of Life and Health Sciences, Aston University, Birmingham

(4) School of Psychology, University of Nottingham, Nottingham

Purpose Driving requires good vision. Retinal diseases and /or cortical damage can cause binocular visual field loss when driving is judged to be unsafe. The current procedures include the HFA and "surrogate" measures of driving performance i.e. Hazard Perception (HP) and Useful Field of Vision (UFOV) tests. The decision to allow driving is based on the binocular Esterman test. We report results of an extensive investigation that examined how loss of visual field sensitivity correlates results derived from HP and UFOV tests in 62 patients and 100 age-matched controls.

Methods The patients tested exhibited a variety of binocular scotomata as a result of either retinal disease or cortical damage. All participants were tested on Esterman, HP, UFOV, a new binocular high density perimetry test and other visual psychophysical tests designed to assess selective loss of visual sensitivity.

Results Both HP and UFOV fail to differentiate patients with a potential problem from normal observers. Loss of visual function is often found well outside the dense area of a scotoma. Significant loss of contrast acuity, motion or colour can be demonstrated in areas of the visual field classified by conventional perimetry as "normal".

Conclusion This study fails to reveal significant correlations between loss of visual field sensitivity and the two surrogate measures of driving performance. The results suggest that driving safety cannot be assessed reliably using current tests. What is needed is an interactive test that examines the combined effects of visual sensitivity loss, distributed attention, eye-movements, decision times and speed of motor response.

■ 4366

Driving and vision: working towards a policy based on evidence

PLANT G

Institute of Neurology, University College, London

Purpose To review the published evidence relating driving performance to visual impairment.

Methods Clinical review.

Results A number of studies have been carried out which attempt to set the limits of visual acuity and visual field for driving. Simulation studies, studies reporting the accident rates of cases with various degrees of visual loss and studies in which on-road driving assessments have been carried out will be reviewed. An association between peripheral field loss and increased crash frequency has been found by some investigators but not others. Studies using simulators or on-road testing have shown individual differences in cases with similar visual field loss.

Conclusion The evidence based on loss of visual acuity and visual field is not likely to be helpful because it does not take into account the ability of the cases to adopt adaptive strategies and neither does it take account of any visual impairment within the peripherically normal areas of the visual field.

■ 4411

Lacrimal film stability

GOES FJR

Goes Eye Centre, Antwerp

Purpose In recent years, both the prevalence and awareness of dry eye have increased significantly. As the frequency of dry eye has progressed, so has our understanding of its underlying pathophysiology, diagnosis and therapeutic approaches. Achieving tear film stability is one of the primary objectives in the treatment of dry eye, as the precorneal tear film forms a continuous cover over the corneal surface between blinks, leading to ocular surface protection. One of the most useful tests to diagnose and monitor dry eye is tear film break-up time. In this study, tear film break up time is used to test the ability of available lubricating eye drops in Belgium to stabilize the tear film, protecting the ocular surface to ultimately provide long lasting relief from the symptoms of dryness experienced by patients.

■ 4412

Lacrimal punctal plugs: new concepts, indications, when and how?

BREMOND GIGNAC D

Pediatric Ophthalmology Department, Robert Debré Hospital, APHP, INSERM U598, Paris

Purpose to provide an overview of lacrimal plugs treatment modalities for dry eye disorders and associated lacrimal substitute.

Methods dry eye syndrome is responsible for chronic and severe impairment of quality of life. When instillation of a lacrimal supplement does not improve dry eye symptoms, lacrimal duct occlusion is used to limit the drainage of tears through the lacrimal ducts. The punctal plug insertion is the most common and conservative method used. Use of lacrimal supplement aims to relieve symptoms by reducing aqueous deficiency or excessive evaporative loss. Recently, a new formulation has been developed that contains polymers of PG 400 and PEG. This innovative formulation also contains polymer hydroxypropyl guar, which acts as a gelling agent in situ. When the hydroxypropyl guar comes into contact with the ocular surface and tears, the neutral pH causes a change in the guar's physical properties and a soft gel forms over the eye increasing viscosity and bioadhesive properties. This promotes the retention of the two demulcents and serves as a protective coating over the ocular surface environment.

Results Plugs are available today in different types of silicone and range in diameter. The plug insertion process consists of determining the appropriate size of plug to insert, dilating the punctum and then inserting the plug with the injector. Granulation is the most commonly observed complication.

Conclusion Successful plug insertion for improving corneal and epithelial disorders consists of: using plugs that are easy to insert and making sure they do not migrate into the lacrimal ducts. The use of plugs in dry eye syndrome must be combined with a high viscosity tear substitute in order to obtain optimal results.

■ 4413

Protecting the Ocular Surface and improving the quality of life of dry eye patients. A comparative study of the efficacy of two tear substitutes in a population of dry eyes

ROLANDO M, AUTORIS S, BADINO M

Department of Neurosciences, Ophthalmology & Genetics, University of Genoa, Genoa

Purpose Tear substitutes are mainly palliative and are aimed to reduce symptoms of discomfort and so to improve the quality of life of the patient. Furthermore the treatment aims to develop a physical-chemical protective barrier in order to limit the epithelial damage to the ocular surface structures typical of this disease.

Methods By means of a prospective, randomised, controlled, parallel study, the effect of two non Newtonian tear substitutes (0.18% HP Guar PEG 400 and PG based and 0.2% Hyaluronic Acid) on the ocular surface staining, interblink tear film stability (Ocular Protection Index) and subjective symptoms was evaluated in 40 patients with diagnosis of moderate to severe dry eye [made on the basis of Symptoms, Schirmer test < 7mm/5' Corneal staining>4 (NEI-I)].

Results Both treatments were able to achieve a statistical significant improvement in symptoms, global corneal staining and OPI, which continued to grow during the time span of the study. 0.18% HP Guar PEG 400 and PG based tear substitute was, with time, more effective than 0.2% Hyaluronic Acid in improving symptoms, global corneal staining and OPI (t-test). When the slope of improvement across time was analyzed, the 0.18% HP Guar PEG 400 and PG based tear substitute showed to improve symptoms and global corneal staining faster than the 0.2% Hyaluronic Acid tear substitute.

Conclusion Both preparations are useful in the palliative treatment of dry eyes. 0.18% HP Guar PEG 400 and PG based treatment demonstrated to be more effective and rapid than 0.2% Hyaluronic Acid in improving symptoms and exerting a protective effect against ocular surface damage shown by staining.

■ 4414

Outcome Analysis of HP Guar PEG 400 and PG based lubricant eye drops in lasik Patients

MERAYO-LLOVES JM

Instituto Universitario de Oftalmobiología Aplicada (IOBA), Universidad de Valladolid, Valladolid

Purpose To study the pathology as well as clinical and psychometric outcomes when using HP Guar PEG 400 and PG based lubricant eye drops to reduce dry eye sensation after lasik.

Methods 10 consecutive patients who underwent lasik for myopia (-1 to -6 D) were instructed to administer lubricant eye drops qid for 1 month after surgery. The following parameters were analyzed before and 1 month after lasik: Psychometric Questionnaire for dry eye, Schirmer test, rose bengale and fluorescein staining and impression cytology.

Results Patients complained about dry eye sensation after lasik (score of the psychometric questionnaire rose from 5.5 to 11). HP Guar PEG 400 and PG based lubricant eye drops reduced the sensation of dry eye. No differences were found in corneal staining before and after surgery. BUT was reduced from 6.6 seconds to 5.5 seconds. No differences were found in the Schirmer test values. Impression cytology evidence showed no changes in the Nelson classification and a reduction of goblet cells were found after lasik.

Conclusion HP Guar PEG 400 and PG based lubricant eye drops after lasik reduce dry eye sensation. Longer follow up, increasing the number of patients, evaluation of impression cytology and the measurements of other parameters like corneal sensation may give more consistent data about the advantages of the use of HP Guar PEG 400 and PG based lubricant eye drops.

■ 4431

Tumour cell vaccines to prevent growth of uveal melanoma

KSANDER BR (1), BOSCH JJ (2), THOMPSON JA (2), OSTRAND-ROSENBERG S (2)

(1) Harvard Medical School, Boston

(2) Dept of Biological Sciences, Univ of Maryland, Baltimore

Purpose Although a number of treatments successfully control the growth of uveal melanomas, no treatment protects patients from liver metastases. We are developing a genetically modified tumor cell vaccine that will generate protective anti-tumor immunity against metastatic tumors. Tumor-specific CD4+ T helper (Th) cells that recognize tumor antigens presented via Class II are known to be essential for anti-tumor immunity. Our data indicate that Invariant Chain blocks Class II from presenting endogenous tumor antigens. We hypothesize that Invariant Chain negative uveal melanomas that express Class II and CD80 will present endogenous antigens and activate Th cells.

Methods Invariant chain negative uveal melanoma cell lines were transfected with retroviral vectors containing: (i) Class II alpha and beta chains and (ii) CD80. Class II matched peripheral blood lymphocytes from healthy donors were restimulated in vitro with either (i) genetically modified, or (ii) unmodified tumor cells. Activation of Th cells was determined via secretion of IFN-gamma using an ELISA.

Results Class II and CD80 positive uveal melanoma cells successfully activated Th cells to secrete IFN-g. Th cells were not activated in the absence of either Class II, or CD80. Although genetically modified primary and metastatic tumor cell lines were both successful in activating Th cells, significantly more IFN-gamma was triggered by primary uveal melanomas. Tumor cells from one patient expressed shared tumor antigens found on a variety of tumors.

Conclusion Genetically modified uveal melanoma cells that express Class II and CD80 present endogenous tumor antigens and activate CD4+ T helper cells in vitro, implying this vaccine may induce protective anti-tumor immunity in vivo.

■ 4432

Using FasL to inhibit tumour growth

GREGORY M (1), SAFFR (2), MARSHAK-ROTHSTEIN A (2), KSANDER BR (1)

(1) Harvard Medical School, Boston

(2) Boston University School of Medicine, Boston

Purpose Fas Ligand contributes to immune privilege by triggering apoptosis of Fas receptor positive infiltrating lymphocytes. However, an alternative function of the membrane form of FasL (mFasL) is to activate Fas positive cells that mediate innate immunity. We propose a novel immunotherapy for ocular tumors using microvesicles expressing mFasL to: terminate ocular immune privilege, reject the tumor, and induce systemic protection from metastases.

Methods DBA/2 mice received an anterior chamber injection of L5178Y-R lymphoma cells expressing (i) wild-type FasL, (ii) soluble-only FasL, (iii) low mFasL, or (iv) high mFasL. A second group of DBA/2 mice received a co-injection of L5178Y-R tumor cells and microvesicles expressing high mFasL into the anterior chamber. Tumor growth and rejection was assessed by slit lamp examination. Inflammation was assessed by histology and immunofluorescence.

Results Tumor cells expressing wild-type or soluble-only FasL grew progressively in the eye and all mice succumbed to metastatic disease. Interestingly, while tumor cells expressing low or high mFasL induced protection from metastatic disease, only mFasL high tumor cells induced a potent inflammatory response, resulting in both rejection of the ocular tumor and systemic protection. Microvesicles expressing high mFasL co-injected with L5178Y-R tumor cells significantly improved survival and induced a potent inflammatory response containing neutrophils and macrophages.

Conclusion We conclude that there is a threshold level of mFasL required to terminate ocular immune privilege. Moreover, bioactive microvesicles can successfully deliver mFasL to an ocular tumor and induce a potent inflammatory response that terminates immune privilege, eliminates ocular tumors, and prevents metastatic disease.

■ 4433

Immunomodulation of intraocular tumours

JAGER MJ (1), BOONMAN ZFHM (1), VAN MIERLO GJD (2), FRANSEN MF (3), MELIEF CJM (3), TOES REM (2)

(1) Ophthalmology, Leiden

(2) Rheumatology, Leiden

(3) Immunohaematology, Leiden

Purpose Tumor cells that are rejected elsewhere in the body, may grow inside the eye. Although intraocular tumor antigens are constitutively presented in the local draining lymph nodes, a systemic CTL response does not develop. Nature provided us with an experiment that showed how natural tolerance can be broken: in some mice, spontaneous shrinkage of the tumor-containing eye (phthisis) resulted after progressive intraocular tumor growth. These mice showed an aberrant immune response.

Methods C57Bl/6 mice received an injection of Ad5E1 plus EJ-ras cells in the anterior chamber of the eye. Specific CFSE labelled T cells were used as indicators for the presence of antigen after adoptive transfer.

Results In about 60% of mice, tumor growth in the eye led to massive infiltration, followed by phthisis: shrinkage of the eye. In non-phthisical tumor eyes, endogenous E1A-specific CTLs were observed only in the draining lymph nodes; in mice with phthisis, CTLs were also present in the spleen, tumor and NDLN. The presence of systemic immunisation prevented the otherwise normal outgrowth of subcutaneously injected Ad5E1A plus ras-transformed tumor cells, while the mice with an intraocular tumor without phthisis were no longer able to reject any Ad5E1A cells, indicating the development of tolerance.

Conclusion Recognition of antigens in a non-inflammatory environment has been postulated to induce tolerance, whereas antigen recognition in a pro-inflammatory context will lead to immunity. The induction of phthisis induced a pro-inflammatory context and a strong induction of systemic immunity, as well as a loss of tolerance. Boonman ZFHM et al. Maintenance of immune tolerance depends on normal tissue homeostasis. J Immunol 2005, 175, 4247-4254.

■ 4434

Presence and phenotype of dendritic cells in uveal melanomas

POLAK ME (1), BORTHWICK NJ (2), JAGER MJ (3), CREE IA (1)

(1) Translational Oncology Research Centre, Portsmouth

(2) Department of Pathology, Institute of Ophthalmology, London

(3) Leiden University Medical Centre, Leiden

Purpose Dendritic cells are potent antigen-presenting cells, capable of initiating an antigen-specific immune response. In cutaneous melanoma, however, DCs were shown to modulate immune response in favour of tumour survival and dissemination. We therefore decided to examine the presence and phenotype of dendritic cells observed within uveal melanoma.

Methods Materials and methods: We assessed expression of FXIIIa, CD68, HLADR, CD40, CD83, TGFbR1, IDO by IHC and ILT3, ILT4, TGFb1, TGFb2 by RT-PCR in 10 paraffin-embedded uveal melanoma specimens. Analysis was performed by microscopy and digital image analysis.

Results Results: We found high numbers of CD68+ APCs in all cases, and FXIIIa positive DCs in 7 out of 10 cases. Both CD68 and FXIIIa cells were HLA-DR positive, FXIIIa cells were however much more dendritic in shape than CD68 cells. Expression of CD40 was detected in occasional cells only, and we found cells expressing CD83 only in one case, which had an extended tumour necrosis. In cases expressing FXIIIa we also detected expression of ILT3. TGFb1 and TGFb2 were ubiquitously expressed in all melanomas, and in all cases the immune cells strongly expressed TGFbR1.

Conclusion Conclusion: Dendritic cells present in uveal melanoma are probably of a tolerogenic phenotype, and as such they can possibly contribute to the immuno-suppressed character of the site. Since the presence of mature DC was however associated with tumour necrosis in one examined case, the anti-melanoma function of dendritic cells is worth further investigation.

■ 4435

IFNg and uveal melanoma resistance to CTL

HALLERMALM K (1, 2, 3), SEKIKI (1), DEGEER A (1), ZUBER B (4), MOTYKA B (5), BLEACKLEY RC (5), JAGER MJ (6), FROELICH CJ (7), KISSLING R (1), LEVITSKY V (2), LEVITSKAYA J (1)

(1) Cancer Center Karolinska, Stockholm

(2) Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm

(3) Swedish Institute for Infectious Disease Control, Stockholm

(4) Mabtech AB, Stockholm

(5) Department of Biochemistry, University of Alberta, Edmonton, Alberta

(6) Department of Ophthalmology, Leiden University Medical Center, Leiden

(7) Department of Medicine, Evanston Northwestern Healthcare Research Institute, Evanston

Purpose IFNg, a pleiotropic cytokine and potent enhancer of antigen presentation to cytotoxic T-lymphocytes (CTL), has been implicated in the control of tumor growth. However, in uveal melanoma patients, elevated serum levels of IFNg and increased expression of HLA class I correlate with spread of metastasis and were suggested as negative prognostic markers. We therefore investigated the effects of IFNg on the MHC class I antigen presentation and susceptibility of uveal melanoma cells to CTL-induced apoptosis.

Results IFNg-treatment enhanced the expression of various components of the antigen presentation machinery, including surface HLA class I, in uveal melanoma cell lines but led to decreased tumor lysis by MHC class I-restricted CTL. This reduction was not due to alterations in CTL activation or impairment in T cell effector functions as measured by cytokine production or degranulation efficiency of CTL. Instead, IFNg-treatment rendered tumour cells resistant to permeabilization by perforin and decreased their capacity to bind and incorporate granzyme B. In accordance, decreased processing of Bid, a substrate of granzyme B, was observed upon tumour cell exposure to antigen-specific CTL.

Conclusion Our data demonstrate that, paradoxically, susceptibility of tumour cells to granule-mediated lysis by CTL can be inhibited by a pro-inflammatory cytokine. This suggests that IFNg can actively impose an immunologically resistant phenotype in tumour cells.

■ 4436

Adjuvant therapy for uveal melanoma

ECONOMOU M (1, 2)

(1) St Eriks Eye Hospital, Stockholm

(2) Karolinska Institute, Stockholm

Purpose To give an overview of the types of treatment currently available as adjuvant therapy for uveal melanoma and discuss new treatment modalities.

Methods Literature review and personal experience of treatments used as adjuvant therapy for uveal melanoma either experimentally *in vivo* or clinically.

Results Immunotherapy with interleukin-2 or interferon (INF) does not show any consistent activity in human metastatic uveal melanoma. Systemic chemotherapy of metastatic disease with bleomycin, vincristine, lomustine and dacarbazine (BOLD) in combination with recombinant interferon 2b have been disappointing. Pre-enucleation irradiation does not affect the survival of patients with uveal melanoma. Intra-arterial fotemustine can induce regression of hepatic metastases but without significant effect on survival time. Other results suggest that carboplatin or chemoembolization with cisplatin may prolong survival. Treatment with temozolamide is largely unsuccessful. Surgical resection of an isolated hepatic metastasis can sometimes prolong life significantly

Conclusion Adjuvant therapy at the time of diagnosis and treatment of the primary tumour generally offers the best chances for improved survival, but this has not been the case for uveal melanoma. Although the new diagnostic and therapeutic tools developed during the most recent years have improved the eye conservation rate, improved survival is rarely achieved. Promising new cancer therapies have been developed, specifically drugs suppressing tumour angiogenesis, and these merit further investigation. Unfortunately, the study of adjuvant therapy for uveal melanoma in the setting of a randomized clinical trial is difficult due to the small number of patients available. This calls for the launch of international multicenter trials.

■ 4441**Dietary Superoxide Dismutase Protects Against Light-Induced Retinal Oxidative Stress in Young Senescence Accelerated Mice (SAM)**

BRON AM (1, 2), SICARD P (3), ACAR N (2), JOFFRE C (2), VERGELY C (3), CREUZOT-GARCHER C (1, 2), BRETILLON L (2), ROCHE TTE L (3)

(1) *Ophthalmology, Dijon*

(2) *Eye and Nutrition Research Group, National Institute for Research on Agronomy, Dijon*

(3) *Laboratory of Experimental Cardiovascular Pathophysiology and Pharmacology, Dijon*

Purpose The aim of this study was to evaluate the protective effect of dietary supplementation in Superoxide Dismutase (SOD) on light-induced oxidative stress in a mouse model for aging, the senescence-accelerated mouse prone 8 (SAM P8).

Methods Weaning SAM P8 and SAM resistant 1 (SAM R1, controls) were used. Animals were exposed 3 times to light (1900 lux for 7 hours) at 1, 2 and 3 months of age. At 3 months of age and before the last light exposure, animals were treated by gavage with SOD (Glisodine®: 10.8 mg/kg/day) or water during 7 days. The scotopic ERG was then recorded and animals were killed in order to measure 1) plasma antioxidant capacity by electron spin resonance using a spin probe CP-H (1-hydroxy-3-carboxy-pyrrolidine), 2) superoxide anion levels on retinal cryosections using an oxidative fluorescent probe (dihydroethidium, DHE, 10 µM).

Results Light-exposure did not alter the ERG response since a and b wave amplitudes were unchanged in either strain. However, plasma antioxidant capacity was increased by 30% in animals treated by SOD. Superoxide anion levels were increased up to 50% ($p<0.01$) in the ganglion cell layer, and by 300% in the outer nuclear layer ($p<0.01$) in all light-exposed mice as compared to non-exposed animals. Within light-exposed animals, the SOD supplementation significantly reduced the superoxide anion levels ($p<0.05$).

Conclusion These results demonstrate that our light-exposure conditions promote retinal oxidative stress without inducing retinal degeneration. However, these data suggest that dietary SOD supplementation is efficient to limit retinal oxidative stress by increasing plasma antioxidant capacity.

■ 4442**PARP inhibition counteracts diabetes-induced retinal oxidative stress and apoptosis**

OBOROSOVA IG (1), SHIN J (1), DREL VR (1), XU W (2), ZHANG J (2), ALI TK (3), JULIUS U (4), EL-REMEZZY A (5)

(1) *Louisiana State University, Baton Rouge, LA*

(2) *MGI Pharma Inc., Baltimore, MD*

(3) *Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA*

(4) *Technical University of Dresden, Dresden*

(5) *Clinical and Experimental Therapeutics, College of Pharmacy, University of Georgia, Augusta, GA*

Purpose To access the role for poly(ADP-ribose) polymerase activation in oxidative stress and apoptosis in diabetic retina and FFA-exposed retinal pericytes and endothelial cells. FFA are an important player in oxidative stress and apoptosis of vascular cells in diabetes.

Methods Control (C) and STZ-diabetic (D) rats were treated with/without the PARP inhibitors, 1,5-isoquinolinediol (ISO, 3 mgkg⁻¹·d⁻¹ i.p.) or 10-(4-Methyl-piperazin-1-ylmethyl)-2H-7-oxa-1,2-diaza-benzo[de]-anthracen-3-one (GPI 15427, 30 mgkg⁻¹·d⁻¹), for 10 wks after 2 wks without treatment. The rate of apoptosis was assessed in flat-mounted retinas by TUNEL assay with immunoperoxidase staining. Primary bovine retinal pericytes and endothelial cells were cultured with/without 0.6 M palmitate, for 48 h. Apoptosis was assessed by TUNEL and caspase-3 assays, superoxide production by ethidium fluorescence, cell viability with Trypan blue, and nitrotyrosine (NT) and poly(ADP-ribose) (PAR) by immunocytochemistry.

Results The number of TUNEL-positive nuclei (Mean ± SEM) was increased ~4-fold in D (207 ± 33 vs 49 ± 4 in C, $p < 0.01$), and this increase was completely corrected in D+ISO and D+GPI 15427 (49 ± 15 and 43 ± 7, respectively, $p < 0.01$ vs D). Palmitate, at the 0.2-0.8 M concentrations, dose dependently increased superoxide production and reduced cell viability in cultured retinal cells. GPI 15427, 20 microM, prevented FFA-induced increase in the rate of apoptosis in pericytes (endothelial cell studies are in progress), and alleviated NT and PAR accumulation in both pericytes and endothelial cells.

Conclusion PARP inhibition counteracts oxidative stress and prevents apoptosis in diabetic retina and FFA-exposed retinal pericytes and endothelial cells.

■ 4443**Stokes-Einstein equation and the physiological effects of vitreous surgery**

STEFANSSON E (1), LOFTSSON T (2)

(1) *Ophthalmology, Reykjavik*

(2) *Pharmacy, Reykjavik*

Purpose Use Fick's law and the Stokes-Einstein equation to understand the effect vitrectomy has on diffusion in the vitreous cavity and the physiology of the eye.

Methods The diffusion of a substance is described by Fick's law $F = -D \frac{dc}{dx}$, where F is the flux of the material, D the diffusion coefficient and $\frac{dc}{dx}$ the gradient of the substance over the distance travelled. Stokes and Einstein derived an equation to describe the diffusion coefficient, $D = R T / 6 (3.14) v r N$, where R is molar gas constant, T is temperature Kelvin, v is viscosity of medium, r is radius of diffusing molecule, and N is Avogadro's number.

Results The rate of diffusion of a substance depends inversely on the viscosity of the medium in which the diffusion takes place. Vitreous humour is 400-2000 times more viscous than water. When vitreous gel is replaced by saline (aqueous humour) the viscosity decreases and the diffusion coefficient increases 400-2000 fold. The diffusion of nutrients such as oxygen within the eye is increased inside the vitreous cavity, from well perfused area of retina to ischemic areas and to the lens. At the same time growth factors also diffuse more easily and are cleared from the retina in the vitrectomized eye. This may explain why the retina has less macular edema and neovascularization following vitrectomy. At the same time neovascularization takes place at the iris, which loses oxygen by diffusion to the posterior segment, while diffusion of growth factors increases from retina to iris.

Conclusion Stokes-Einstein equation predicts changes in diffusion characteristics in the vitreous cavity following vitrectomy. The resulting physiological changes have been confirmed experimentally and explain some of the clinical consequences of vitreous surgery.

■ 4444 / 454**Oximetry in retinal vascular occlusions**

HARDARSSON SH (1), KARLSSON RA (2), HALLDORSSON GH (2), JOELSSON SR (2), EYSTEINSSON T (1), BENEDIKTSSON JA (2), BEACH JM (2), STEFANSSON E (1)

(1) *University of Iceland, Ophthalmology, Reykjavik*

(2) *University of Iceland, Electrical and Computer Engineering, Reykjavik*

Purpose To analyze hemoglobin oxygen saturation (SatO₂) in retinal vascular occlusions using an automatic non-invasive oximeter.

Methods The oximeter is composed of a fundus camera, beam splitter and a digital camera. Specialized software analyzes images from the oximeter and calculates the SatO₂ within retinal vessels. Measurements were made on 13 patients with branch retinal vein occlusion (BRVO), 1 with hemivein occlusion, 2 with central retinal vein occlusion (CRVO) and 5 with branch arteriolar occlusion (BRAO).

Results In patients with retinal vein occlusions (RVO, all types) the venous SatO₂ in healthy eyes was 52±13% (mean±SD; n=12), 54±11% in non-affected venules of RVO eyes (n=12), 35±20% in affected venules before laser treatment (n=10) and 53±5% in affected venules after laser treatment (n=8). Before laser treatment, the affected venules had a significantly lower SatO₂ than the non-affected venules ($p<0.01$) or venules in the healthy eyes ($p<0.05$). In the 5 BRVO eyes measured both before and after laser treatment the venous SatO₂ rose from 45% to 53% ($p=0.041$). The SatO₂ in the occluded hemivein (1 case) increased stepwise following addition of laser treatment. Both CRVO eyes were very hypoxic. The SatO₂ was normal in the 5 BRAO eyes except for a hypoxic venule in the area of occlusion in 1 eye (the most recent BRAO).

Conclusion The oximeter shows decreased venous SatO₂ following RVO and a rise in SatO₂ with laser treatment.

■ 4445 / 455

Diabetes-induced cataract amplified in mice lacking CuZn-superoxide dismutase

OLOFSSON EM (1), MARKLUND SL (2), BEHNDIG A (1)

(1) Department of Clinical Sciences/Ophthalmology, Umeå University, Umeå

(2) Department of Medical Biosciences, Clinical Chemistry, Umeå University, Umeå

Purpose Lenses from mice lacking the antioxidant copper-zinc superoxide dismutase (SOD1 null mice) develop cataract and show signs of cell damage when exposed to high levels of glucose in vitro. The superoxide radical, elevated in the SOD1 null lenses, is thought to contribute to the cataractogenesis in these lenses possibly through the reaction with nitric oxide, generating the highly toxic peroxynitrite. In this study, we aimed to evaluate the effect of streptozotocin-induced diabetes mellitus on cataract formation in SOD1 null mice *in vivo*.

Methods SOD1 null and wild-type mice were made diabetic by repeated intraperitoneal streptozotocin injections for 5 consecutive days. Mice injected with citrate buffer were used as controls. After eight weeks of diabetes, the mice were sacrificed and the lenses removed and photographed in retro-illumination. The cataract formation was quantified from the photographs using digital image analysis.

Results After eight weeks, the diabetic SOD1 null mice showed more cataract compared to all the other groups (2-way ANOVA; diabetic*SOD1 null; p=0.001).

Conclusion This *in vivo* study supports our previous *in vitro* studies and gives further evidence on the protective role of SOD1 against diabetic cataract formation.

■ 4446 / 456

Transient Hypertonic Saline (ths)-induced Intraocular Pressure (IOP) as a rabbit model to assess potential new antiglaucoma drugs

BASTIA E, IMPAGNATIELLO F, BORGHINI V, BIONDI S

Nicox Research Institute, Bresso, Milan

Purpose Reducing IOP is the major therapeutic strategy for the treatment of glaucoma. Indeed, various animal models have been described, even though their sensitivity to drug treatment differs considerably, making difficult the selection of new compounds. In this study, we describe the effects of different drugs acting on aqueous humor inflow such as timolol and dorzolamide in THS-induced IOP in NZW rabbits as well as in normotensive NZW and pigmented DB rabbits.

Methods In THS, IOP was raised by the injection of hypertonic saline (5%) into the anterior chamber.

Results Raised IOP reached a peak at 30 min (2-3 fold over basal, 16±2mmHg), stabilized during the following 60 min and decayed gradually in the following 3h. Topical application of timolol significantly and dose-dependently (Δ maxIOP: -3+3, -6+3; -7+2mmHg, at 0.1, 0.3 and 1% respectively) reduced IOP increase, starting 30 min after instillation. Similarly, dorzolamide (2%) lowered IOP (Δ maxIOP: -6+2mmHg) from 30 min after instillation. In DB rabbits, basal IOP was significantly higher compared to NZW (20 vs 16mmHg). Timolol (1%) and dorzolamide (2%) significantly lowered IOP at 60 min after instillation (Δ maxIOP: -5+1; -7+1mmHg, respectively). Conversely, in normotensive NZW rabbits, both timolol and dorzolamide only slightly and not significantly reduced IOP.

Conclusion We show that the THS-induced IOP rabbit model is suitable to detect the IOP-lowering effect of commercially available drugs. Further studies are ongoing to evaluate the potential usefulness of this model for the screening of newly synthesized drugs.

■ 4447 / 457

Invasive Real Intraocular Pressure (IOP) Measurement.**How to do it correctly**

HERNANDEZ VERDEJO JL (1), ROMAN JM (2), BOLIVAR G (3), FERRER B (2),

TEUS MA (2)

(1) VISSUM Corporación oftalmológica., Madrid

(2) VISSUM, Madrid

(3) Universidad de Alcalá, Madrid

Purpose To show an experimental model to demonstrate the correct way to measure real IOP in porcine eyes.

Methods Values of real IOP was measured in freshly enucleated porcine eyes. Two reusable blood pressure transducers were used. The globes were inflated with 5% glucosated solution through the optic nerve to attain an IOP of 10-20 mmHg. A 27-gauge catheter was inserted from pars plana to the anterior chamber and another 27-gauge catheter was inserted into the vitreous cavity with any response, then the vitreous catheter was change for a 21-gauge catheter. Real IOP was then transmitted via the catheter liquid column to both external sensors. At that moment the IOP was increased into the anterior chamber via another catheter connected to a liquid column. IOP changes were registered for a period of 60 seconds.

Results Using the 27-gauge catheter, our model was unable to register changes of IOP in the vitreous chamber (VC). However, when the 21-gauge catheter was used, the transducers detected simultaneously changes of IOP both in the anterior chamber (AC) and in the VC until an IOP of 40 mmHg was reached. Beneath that point, there was a delay in the IOP raise into the VC, so when a level of 80 mmHg was reached into the AC, the transducer measured an IOP 20 mmHg less into the VC. At the end of the measurements IOP values remained stable.

Conclusion Our experimental model demonstrates that cannulation into the vitreous chamber as invasive method to measure IOP is not the correct way to register real IOP.

■ 4451**Long-term result of trabeculectomy with mitomycin C (MMC) using modern advanced techniques**

CHIANG MY, SII F, WILLIAMS TA, LOCKWOOD A, LEE GA, SHAH P
Birmingham and Midland Eye Centre, Birmingham

Purpose To study the long term efficacy and safety profile of trabeculectomy with MMC using modern techniques

Methods The Birmingham ReGAE (Research into Glaucoma And Ethnicity) Project is a multidisciplinary, multiphase and ethnically-sensitive research project to prevent glaucoma blindness. Phase 3 studies the outcome of trabeculectomy with MMC in a prospective and consecutive interventional case series. There are currently 226 eyes in the project.

Results One hundred and fifty-three eyes of 126 patients with 1 year follow-up were included. There were no other exclusion criteria. Mean follow-up duration was 1064.2 days (range 365 - 2240 days). There were 110 Caucasian (71.9%), 33 African-Caribbean (21.6%) and 10 Asian (6.5%) eyes. Fifty-one eyes (33.3%) had previous intraocular surgery and 14 (9.2%) had previous trabeculectomy. Intraocular pressure (IOP) decreased significantly from 23.08 ± 7.33 mmHg to 12.42 ± 3.80 mmHg ($p < 0.05$). Kaplan-Meier survival probabilities were 96.7% at 1 year and 95.8% at 3 years and 5 years using $IOP \leq 21$ mmHg. For $IOP \leq 17$ mmHg, the survival probabilities were 92.2% at 1 year, 88.4% at 3 years and 5 years. Using $IOP \leq 14$ mmHg, the survival probabilities decreased to 79.1% at 1 year, 65.7% at 3 years and 63.1% at 5 years. Visual loss of > 2 Snellen lines associated with surgery occurred in 3 eyes (2.0%). One eye required tube surgery at 1 year (0.65%). Seven eyes (4.6%) had clinically significant early reversible hypotony needing intervention. Two eyes (1.3%) underwent bleb revision due to late hypotony.

Conclusion Trabeculectomy with MMC provided long-term effectiveness and safety profile in our series with complex glaucoma case-mix.

■ 4452**ReGAE 3 (Research into Glaucoma and Ethnicity)**

Trabeculectomy with Mitomycin C (MMC) – a two year follow up
LOCKWOOD A, SII F, CHIANG MY, WILLIAMS TA, SHAH P
Birmingham and Midland Eye Centre, Birmingham

Purpose To compare the efficacy of trabeculectomy with MMC in the African-Caribbean, Asian and Caucasian populations at 2 years.

Methods This is a part of Phase 3 of the Birmingham ReGAE project, an open, consecutive case series of patients who had undergone trabeculectomy with mitomycin C. All patients of African-Caribbean (AFC), Asian and Caucasian backgrounds were included. Statistical analysis was with Chi Squared test.

Results Of 226 eyes (16.3 % AFC, 7.6 % Asian and 76.1 % Caucasian), 106 (22.6 % AFCs, 4.7 % Asian and 72.6 % Caucasian) had a minimum of 2-years follow up. At 2 years those who achieved 21 mm Hg or less were 92 % AFCs, 100 % Asians and 100 % Caucasians. Those who achieved 14 mm Hg 66 % AFCs, 100 % Asians and 84.4 % Caucasians, (AFC vs Caucasian $p = 0.017$). Thirty percent reduction of mean preoperative IOP was achieved by 79 % AFCs, 100 % Asians and 77 % Caucasians. The proportions of AFCs, Asians and Caucasians requiring anti-glaucoma medication postoperatively at 2 years were 29%, 40 % and 6.4 % respectively (Caucasian vs AFC $p = 0.0027$, Caucasian vs Asian $p = 0.0094$). The mean number of medications were 0.74 (AFC), 1.4 (Asian) and 0.08 (Caucasian). Bleb needling revision with 5FU was required in 29 % AFCs, 20 % Asians and 12 % Caucasians. The rates of failure were 3 % AFC, 20 % Asian and 6 % Caucasians.

Conclusion AFC and Asian eyes required more postoperative interventions, both medical and surgical, than Caucasian eyes to achieve IOP reduction.

■ 4453**Morphological evaluation of blebs after filtering glaucoma surgery by means of in vivo confocal microscopy and optical coherence tomography**

LANZINI M, NUBILE M, FASANELLA V, COSTANTINO O, MASTROPASQUA L
Ophthalmic Clinic - University "G. d'Annunzio" Chieti-Pescara, Chieti

Purpose To analyze bleb structure after filtering surgery using in vivo confocal microscopy (IVCM) and anterior segment optical coherence tomography (OCT).

Methods Observational case series. 25 filtering blebs of 20 patients after trabeculectomy were evaluated by using laser scanning IVCM and Visante OCT. Eyes were classified into two groups: (1) functioning blebs (13 eyes) and (2) nonfunctioning blebs (12 eyes), based on tonometric values. Cellular patterns, morphologic appearance, and functional aspects of functioning and nonfunctioning blebs were compared in a masked fashion.

Results IVCM showed that all functioning blebs had numerous conjunctival intraepithelial optically-empty microcysts and spaced reticular subepithelial connective tissue. Conversely all nonfunctioning blebs showed rare conjunctival epithelial microcysts and dense, compact subconjunctival connective tissue. OCT showed that intrascleral lake parameters (area, radial longitudinal length, height of intrascleral space) were significantly greater in functioning blebs ($p < 0.001$). Moreover low reflective subconjunctival empty micro-dots originating from the scleral lake connecting to the conjunctiva were observed in this group.

Conclusion The number of epithelial microcysts and the quality of subconjunctival connective tissue, as observed with IVCM, and the OCT morphological signs of intrascleral and subconjunctival aqueous outflow appeared correlated with bleb function

■ 4454**On table gonioscopy with intraocular viscoelastic in the operating theatre for accurate identification of cyclodialysis cleft in occult hypotony**

WILLIAMS TA, CHIANG YM, LOCKWOOD A, SII F, SHAH P
Birmingham and Midland Eye Centre, Birmingham

Purpose This video demonstrates the technique of on table gonioscopy with intraocular viscoelastic in the operating theatre under topical anaesthesia for accurate identification assessment of cyclodialysis cleft in occult hypotony.

Methods Assessment of the aetiology of occult hypotony may be problematic, the differential diagnosis of which includes cyclodialysis cleft. These clefts can be very difficult to identify on the slit lamp when the eye is hypotonous. As part of the planned surgical management of cyclodialysis cleft, we describe the use of on table gonioscopy using a sterile Zeiss two mirror gonio lens after the anterior chamber has been filled with viscoelastic via a paracentesis under topical anaesthesia.

Results Accurate determination of the extent and location of the cleft and assessment of the morphology of the remaining angle (extent of angle recession, synechiae and angle closure) is illustrated in this video.

Conclusion The technique described in this video to identify cyclodialysis clefts may be pivotal in the management of occult hypotony.

■ 4455

The comparison of outcome results of using diode laser for neovascular glaucoma after diseases of retina

BLINIATYAN IY

Glaucoma, Ophthalmologic Centre n.a. S.V.Malayan, Yerevan

Purpose Several types of glaucoma are associated with diseases of the retina. Most frequent of them are retinal vein occlusion and diabetes mellitus. The aim of our report is to try to compare outcome results of using semiconductor diode laser for neovascular glaucoma-NVG after vein thrombosis and after diabetic retinopathy.

Methods 15 eyes with NVG after vein thrombosis (I) and 15 eyes after diabetic retinopathy (II) were examined in equal conditions: power 1500-2000 mW, duration 1000-1500 msec, number of coagulants 24. All patients were with pain syndrome and had rubeosis (but only along the edges of pupil). The TSCP was performed with IRIS Oculight Diode Photocoagulator with G-probe, Medical Instruments, Inc., with local anesthesia and anti-inflammatory eye drop medications and oral anti-inflammatory medications with usual treatment after.

Results Initial IOP were 44.93 mm Hg for I and 47.87 mm Hg for II. All patients reported of disappearance of pain syndrome. In most cases rubeosis was reduced. Next day after a raise of IOP level was in 6.67% of cases in I and in 20% for II. Observational data for 7, 30, 60, 90 days were approximately equal. And observation after 1 year and more were: I-the patients with high IOP level 13.33%, mildly high level of IOP 46.67% and with normal IOP level- 40%; II-the patients with high IOP level 20%, mildly high level of IOP 26.67% and with normal IOP level - 53.33%.

Conclusion Treatment with G-probe diode laser is efficient method of treatment NVG after retinal vein occlusion and diabetes mellitus. This method leads to eliminating the pain syndrome, reducing the IOP level and rubeosis and keeping the eye as an organ. These parameters are more suitable for NVG after diabetic retinopathy.

■ 4456

Effect of non penetrating glaucoma filtering surgery on visual acuity. A one year follow up study

TELESMA (1, 2), ARRANZ E (2), GIL R (2), PIZZAMIGLIO-MARTIN C (2), MARINA C (2)

(1) University of Alcalá, Madrid

(2) Visum Madrid, Madrid

Purpose To evaluate the effect of non penetrating filtering surgery on visual acuity during a one year follow-up period, in patients with primary open angle glaucoma (POAG).

Methods This is a prospective trial in which non penetrating filtering surgery was performed to 132 POAG eyes, (132 patients). The same surgeon performed all procedures using intraoperative Mytomicine C, and without placing any implant in the scleral bed. Main outcome measures were best corrected visual acuity (BCVA), manifest refraction, Goldmann applanation tonometry and the number of hypotensive treatments pre and postoperatively. In all cases the same examiner obtained the measurements preoperatively and at every follow-up postoperative visit at one day, 1, 3, 6, 9 and 12 months.

Results Snellen BCVA was 0.7 ± 0.3 preoperatively, and 0.69 ± 0.29 at the one month postoperative visit, therefore there was no statistically significant difference on BCVA postoperatively comparing with the preoperative values ($p=0.5$). We found a reduction on IOP ($p=0.001$) at every postoperative follow-up visit. Mean preoperative IOP (mmHg) was 20 ± 5.4 , and postoperatively 12.1 ± 4.2 , 13.4 ± 4.1 , 13.5 ± 3.3 , 14.8 ± 4.1 , 15 ± 4.1 , at 1, 3, 6, 9, and 12 months respectively. The mean number of medications was reduced from 1.7 ± 0.8 preoperatively to 0.1 ± 0.4 at 1 year after deep sclerectomy was performed.

Conclusion Non penetrating filtering surgery achieves a substantial IOP decrease without negatively interfering with visual acuity, in contrast of that previously reported for classic trabeculectomy. These findings suggest an advantage for non penetrating filtering surgery.

■ 4457 / 421

The safety and efficacy of trabeculectomy with mitomycin C (MMC) in juvenile open angle glaucomaCHIANG MY, LOCKWOOD A, WILLIAMS TA, JAYAPRAKASAM A, SHAH P
Birmingham and Midland Eye Centre, Birmingham

Purpose To determine whether trabeculectomy with MMC can be used safely and effectively to control the intraocular pressure (IOP) in patients with severe refractory juvenile open angle glaucoma (JOAG).

Methods This study emerged as part of Phase 3 of the Birmingham ReGAE (Research into Glaucoma And Ethnicity) Project- a prospective and consecutive interventional case series investigating the surgical outcomes of trabeculectomies with MMC. There are currently 226 eyes in this actively updated database. Patients with JOAG were selected for the study.

Results Twenty-three eyes of 15 patients with JOAG were included. Ten out of 23 eyes (43.5%) were African-Caribbean eyes and 5 eyes (21.7%) had previous trabeculectomy. The mean age at surgery was 30.96 (range 11 – 48). The mean duration of follow-up was 633 days (range 90 – 2172 days). Twenty-two eyes (95.7%) achieved IOP of ≤ 21 mmHg at the latest follow-up and 19 eyes (82.6%) did not require any medication. Fourteen eyes reached 1 year follow-up. Success at 1 year stratified by IOP were 92.9% (n=13) at IOP ≤ 21 mmHg and ≤ 17 mmHg and 85.7% (n=12) at IOP 14 mmHg. An IOP reduction of $\geq 30\%$ were obtained in 92.9% (n=13). The mean IOP decreased significantly from 23.36 ± 2.69 mmHg pre-operatively to 13.21 ± 1.51 mmHg at 1 year ($p<0.05$). No visual loss of > 2 Snellen lines occurred. Three eyes (13%) had clinically significant early hypotony needing intervention. One eye (4.3%) developed a late bleb leak requiring bleb revision. One eye (4.3%) required tube surgery at 1 year.

Conclusion Trabeculectomy with MMC can be used safely and effectively to control IOP and preserve vision in refractory juvenile open angle glaucoma.

■ 4458 / 423

Excimer laser trabeculotomy (ELT) - an alternative laser treatment to reduce intraocular pressure

WILMSMEYER S (1), PACHE M (1), FUNK J (2)

(1) University Eye Hospital, Freiburg

(2) Charlottenklinik, Stuttgart

Purpose Excimer laser trabeculotomy (ELT) is a minimally invasive surgical technique to reduce intraocular pressure (IOP) in patients with glaucoma or ocular hypertension. Our purpose was to examine IOP-reduction in patients treated with ELT alone or ELT combined with cataract surgery.

Methods To increase the outflow of aqueous humor, 10 microperforations of the trabecular meshwork were performed by an endoscope guided photoablative laser probe (Excimer laser, AIDA, TUI-Laser, Munich). In our retrospective study one group of patients received an ELT alone, the other group underwent combined surgery consisting of phacoemulsification + ELT. IOP, visual acuity and antiglaucoma drugs (AGD) were determined preoperatively (T0) and 2-4 (T1), 5-7 (T2), 10-14 (T3) and 21-27 (T4) months after surgery.

Results IOP in eyes with ELT alone was reduced from 24.1 (n=69) mmHg preoperatively to 18.8 (T1, n=67), 19.9 (T2, n=60), 18.9 (T3, n=49), 19.4 (T4, n=43) mmHg resp.. The number of AGD was 1.9 (T0), 1.2 (T1), 1.4 (T2), 1.9 (T3), 1.4 (T4). In the phacoemulsification + ELT group, an IOP-reduction from 22.4 mmHg (T0, n=58) to 16.5 (T1, n=55), 16.2 (T2, n=49), 16.2 (T3, n=51), 15.6 (T4, n=38) mmHg resp. was observed. The number of AGD showed no significant change (1.2 (T0), 0.9 (T1), 1.1 (T2), 1.2 (T3), 1.1 (T4)).

Conclusion ELT, especially in combination with phacoemulsification, is a promising laser treatment to reduce IOP for at least 1-2 years.

■ 4461

Use of Electrophysiologic Testing in Everyday Practice

LAM BL

Bascom Palmer Eye Institute, Miami

Purpose To provide an overview of the clinical utility of electrophysiologic tests.**Methods** Case examples from an active neuro-ophthalmologic and electrophysiologic service.**Results** Clinical indications of visual electrophysiologic testing are evolving with the development of new electrophysiologic techniques. Electrophysiologic tests should be selected and considered on an individual basis in patients based on history and clinical findings. More common clinical electrophysiologic testing include the full-field electroretinogram (ERG), multifocal ERG, pattern ERG, electro-oculogram (EOG), pattern visual evoked potential (VEP), and multifocal VEP.**Conclusion** A basic understanding of visual electrophysiologic tests and their clinical indications enables the clinician to appropriately select the type of electrophysiologic testing necessary in everyday practice

■ 4462

Functional imaging of the retina

HAWLINA M

University Eye Clinic, Ljubljana

ABSTRACT NOT PROVIDED

■ 4463

Using the EOG as a detective tool

LEROY BP (1, 2), YARDLEY J (3), HART-HOLDEN N (3), LAFAUT BA (4),
 PUIECH B (5), DE LAEY JJ (1), MOORE AT (6), KESTELYN P (7), BLACK GCM (3, 8)
 (1) Department of Ophthalmology, Ghent University Hospital, Ghent
 (2) Center for Medical Genetics, Ghent University Hospital, Ghent
 (3) Academic Unit of Medical Genetics & Regional Genetics Service, St Mary's Hospital, Manchester
 (4) Department of Ophthalmology, St Jan Hospital, Bruges
 (5) Exploration Fonctionnelle de la Vision, CHRU Hôpital Roger Salengro, Lille
 (6) Department of Molecular Genetics, Institute of Ophthalmology, London
 (7) Department of Ophthalmology, Ghent
 (8) Academic Department of Ophthalmology, Manchester Royal Eye Hospital, Manchester

Purpose To illustrate the role of electro-oculography in the elucidation of the pathogenetic mechanism in autosomal dominant vitreoretinochoroidopathy (ADVICR).**Methods** Forty-one affected individuals of 5 large ADVIRC families were clinically examined. In the majority of these additional electrophysiological evaluation was performed, including electro-oculography (EOG) and electroretinography (ERG). Linkage analysis suggested that the gene responsible for ADVIRC is located on chromosome 11q12.3. Abnormal EOGs early in the course of the disease suggested that the RPE-specific gene VMD2 was the prime candidate. Mutation detection analysis in the VMD2 gene was performed in all patients.**Results** All patients (100%) had the characteristic hyperpigmented retinal band from equator to ora serrata. In 83% of them microcornea was noted. About 56% of patients had vitreous abnormalities, cells in the vitreous were seen in 25%. In 54% of patients the anterior chamber was shallow. A significantly reduced Arden-De Rouck ratio on EOG was noted in 100% of patients, very often well before ERG responses were reduced. The ERG was abnormal in 77% of patients. Mutations in VMD2 were identified in all 5 families, which result in simultaneous missense substitutions and exon skipping in each individual.**Conclusion** The EOG is abnormal in a subset of ADVIRC patients, early during the course of the disease and prior to ERG abnormalities, suggesting that the RPE is the primary site of disease. Indeed, mutations in VMD2 are the underlying cause in ADVIRC. The clinical phenotype of ADVIRC is that of a variably severe chorioretinal dystrophy in combination with ocular developmental defects.

■ 4464

The value of Visual Evoked Potentials (VEP) in the monitoring and prognosis of pituitary tumours.

GRZYBOWSKI AE (1, 2), LIEBERT W (3), ADAMCZAK N (4), DRUZDZA (5), MISKOWIAK B (4)

(1) Electrophysiology Department, City Hospital Nowe Miasto, Poznan
 (2) Department of History of Medicine, Poznan University of Medical Sciences, Poznan
 (3) Department of Neuro-surgery, Poznan University of Medical Sciences, Poznan
 (4) Department of Optometry and Biology of Visual System, Poznan University of Medical Sciences, Poznan
 (5) Department of Neurology, Military Hospital, Poznan

Purpose In more than 80% of pituitary tumor patients visual acuity impairment and/or visual field deterioration are the first noticed signs of the disease. Thus, the functional tests of the visual system are of growing interest. The aim of the study was to: 1.compare the ophthalmological and the electrophysiological methods in pituitary tumor patients before and after an operation; 2.determine the relations between the deterioration of the visual system and the size and location of a tumor; 3.assess the usefulness of VEPs as a monitoring and prognostic tool in pituitary tumors.**Methods** 29 consecutive patients with pituitary tumors were included in the clinical programme involving a detailed ophthalmology examination and electrodiagnostic tests. 20 patients were operated. The detailed eye examination consisted of visual acuity measurement, slit lamp examination of anterior and posterior segment, static perimetry, panel D-15 and Ishihara plate colour vision tests. The electrodiagnostic tests consisted of different modes of VEPs, including pattern reversal, pattern flash the half-field and quadrant-field stimulation of visual evoked potentials. The electrodiagnostic tests and ophthalmology examinations were performed in the group of operated patients one month and six months after the operation.**Results** The results of ophthalmology examination and electrodiagnostic tests were correlated with the size of the tumor, its histopathology structure and Hardy-Wilson classification. In the group of operated patients the results of the ophthalmology examination and electrodiagnostic tests in different time intervals were compared and correlated by statistical methods.

■ 4465

Diagnostic value of electroretinography in retinitis pigmentosa associated syndromes

FARKAS A

Semmelweis University, Department of Ophthalmology, Budapest

Purpose Retinitis pigmentosa (RP) is a well-defined clinical condition and in the majority of cases, electroretinography (ERG) is only required to confirm the diagnosis. In syndromes related to RP, symptoms of other affected organs are dominant in childhood while the symptoms related to the eye become suddenly apparent around puberty. The aim of the present work is to give an overview of the most common syndromes associated with RP, based on the clinical cases in our department.

Methods 40 patients with sensorineural hearing loss, 5 patients with endocrine disorders, slight mental retardation and additional digits at birth and, 3 patients with renal insufficiency from early childhood were ophthalmologically tested. The examination protocol included visual acuity, fields, fundoscopy and ERG. In certain cases molecular genetic tests were also performed in institutions outside Hungary.

Results Decreased visual acuity, concentric field defects and extinguished or, in some cases, markedly subnormal ERGs were found. In the fundi pale optic nerve head, narrow vessels and retinal pigmentation of different character were seen. Based on the above data clinical diagnosis of Usher syndrome, Bardet-Biedl syndrome and Senior-Loken (?) syndrome were concluded.

Conclusion In cases of obscure general conditions, ophthalmological examinations, including ERG (under general anaesthesia, if requested) is important in order to confirm the diagnosis. An early precise diagnosis enables the patients and their families to prepare for the future.

■ 4466

Cone dystrophy with supernormal rod ERG

HOLDER GE (1), MICHAELIDES M (1, 2), WUH (1, 2), ROBSON AG (1),

JENKINS S (1, 2), MOORE AT (1, 2), HUNT DM (2), WEBSTER AR (1, 2)

(1) Moorfields Eye Hospital, London

(2) Institute of Ophthalmology, London

Purpose "Cone dystrophy with supernormal rod ERG (CDSR)" is a rare recessively inherited retinal degeneration. The purpose of the study was to describe the phenotype and seek the gene responsible for the disorder.

Methods Ten patients with CDSR were ascertained electrophysiologically using protocols that exceeded but incorporated the ISCEV Standard ERGs and PERG. In particular, an ERG intensity series and ON/OFF response recording were performed in most patients. Blood was taken and DNA extracted for mutational screening.

Results All patients showed the characteristic ERG findings. There was a very delayed rod specific ERG of low amplitude that showed large changes over a relatively small range of stimulus intensities; the bright flash ERG was of unusual waveform with a normally developing a-wave followed by a broadened a-wave trough and a very high amplitude, sharply rising b-wave; cone single flash and flicker ERGs were delayed and subnormal. All patients in whom ON and OFF response data were available showed delayed OFF responses.

Conclusion The data available from DNA screening will be presented. The nature of the characteristic ERG changes that occur in the disorder will be discussed.



2006 | October 4 - 7, 2006
Vilamoura, Portugal

www.ever.be

**ALL
AUTHORS
INDEX**

All authors of abstracts are listed alphabetically.

Three digit numbers refer to posters.

Four digit numbers refer to oral presentations.

The digit numbers marked in bold indicate a first author abstract.

ABAHUSSIN M: 201, 2336	AUTORI S: 4413	BENEDIKTSSON JA: 454, 4252, 4444
ABEDIN A: 2233	AUTRATA R: 232, 2343	BENEZRA D: 352
ACAR N: 4441	AYALA M: 227, 2144, 2344	BENGSSON B: 4151
ACQUART S: 475	AYDIN P: 1344, 3224 , 3126	BENITO Y: 2124
ACQUAVIVA A: 4333	BAARSMA GS: 220, 2322	BENTO CA: 205
ADAMCZAK N: 4464	BACANLI D: 4242	BERAKI K: 351
ADAMIS AP: 3121	BACH M: 3441	BERGMANN F: 218
ADENIS JP: 337, 372, 3157	BACHMANN B: 356, 338, 3158	BERGMANN M: 3112
AGOSTINI HT: 376, 4223 , 3143, 3216	BACKHAUS J: 437, 2257	BERGUA A: 216
AGUSSAN J: 234	BACKLUND T: 2254	BERISHA F: 447, 3427, 4344 , 3423, 3424
AKGUEL H: 4336	BADALA F: 2452	BERNARDES R: 2167 , 2163, 2164, 3366
AKHTAR S: 209, 2332	BADINO M: 4413	BERROD JP: 3264
AKITA J: 3121	BAKSHINSKI P: 411	BERTELMANN E: 331, 2137
AKKOYUN I: 304, 4242	BAKUNOWICZ-LAZARCZYK A: 350	BERTUZZI F: 4153
AKOVA YA: 304, 4242	BALESTRAZZI A: 358	BESCH D: 3444
ALARBI M: 215, 2126	BALMER A: 2433	BEST R: 215, 2126
ALEXIOU-DANIEL S: 471	BANDELLO F: 3464	BHATTACHARYA SS: 3163 , 2111
ALI N: 317	BANKS EA: 3242	BIALLOSTERSKI C: 2162
ALI RR: 3166	BAPTISTA A: 473	BICHÓ MP: 4321 , 4324
ALI TK: 4442	BAPTISTA P: 2163 , 2164	BIEBACK K: 3144
ALIAS EG: 434, 432	BARAIBAR B: 320, 427	BINI A: 380
ALIO JL: 339, 368, 2445 , 3159	BARAK V: 3335	BIONDI S: 456, 4446
ALJAMAL R: 3435	BARBOSA A: 2166	BIRD AC: 2465, 3322, 3361
ALLAN E: 4231	BARBUR JL: 3443 , 3447, 4363, 4364, 4365	BISCOE MP: 3142
ALMARAZ A: 485, 3331	BARCIA A: 367, 2133	BITTI A: 217
ALMEIDA JB: 363, 440	BARCIA RN: 364, 2131	BIZIOS D: 4151
ALOE L: 340, 3355	BARNSTABLE CJ: 2313 , 2311	BLACK GCM: 4463
ALSPAC STUDYGROUP: 2212	BARRAQUER J: 2342	BLANC D: 352
ALTHAUSER S: 412, 2456	BARRAQUER RI: 332, 2446 , 2138	BLANCHON L: 202, 2337
ALVANOU A: 336, 3156	BARRY J-S: 325	BLANCO G: 480, 483, 485, 3432
ALVAREZ BV: 242	BARTESELLI G: 321 , 2462	BLANCO-MEZQUITTA JT: 367, 3353 , 2133
ALVAREZ MT: 319, 369	BARTZ-SCHMIDT KU: 3444	BLEACKLEY RC: 4435
ALVES-FARIA P: 470	BASTIA E: 456, 4446	BLUMENKRANZ MS: 3263
AL YOUSSEF A: 372	BASTOS A: 2123	BLUMENTHAL EZ: 4334
AMAT P: 469	BATEMAN S: 341, 3356	BOCCASSINI B: 3333
AMATI-BONNEAU P: 3411 , 3315, 3412	BATES D: 4221	BOCK A: 4342
AMIRIDIS I: 225	BATRA R: 302, 3266	BOCK FB: 338, 356, 3158
AMIRYAN AG: 478, 3438	BAUDOUIN C: 335, 3155	BODAGHI B: 1325, 2121, 2221
ANDERSSON AM: 244	BAUER C: 206	BOGDANICI C: 481
ANDREEVA LD: 256	BAUER SM: 2154	BOLIVAR G: 464 , 326, 420, 457, 463, 4447
ANDRES M: 3441	BAUMGART J: 3262	BOLLET M: 3132
ANSARI EA: 317 , 419	BAYON A: 4243	BOMBARDIERI CB: 212
ANTON B: 258	BEACH JM: 454, 4252, 4444	BONAIUTI M: 453, 4347
ANTONIADIS M: 225	BEATTY S: 2243	BONINI S: 3353
ANWAR ST: 3153	BECKER MD: 2325	BONNEAU D: 3315, 3411, 3412
APPELT C: 330, 2136	BEDANE C: 372	BONNET D: 2312
ARENDO: 2252	BEER M: 3165	BOONMAN ZFHM: 4433
ARNDT C: 3315	BEHAR-COHEN F: 352, 468	BOOTH A: 325
ARRANZ E: 3451, 4456	BEHNDIG A: 455, 4445	BORDIN M: 3122
ARTAL P: 2442	BEJJANI BA: 375, 2417	BORGES DE ALMEIDA J: 254, 3445
ARTOLA A: 339, 3159	BEJJANI RA: 352	BORGHINI V: 456, 4446
ASSELAIN B: 2362, 3135, 4331	BEKIR NA: 462	BORNFELD N: 4336
Association of Vision Science Librarians: 257, 258	BELENGUER P: 3315	BORQUE E: 435 , 208, 425, 4155 , 4154
ASSOULINE M: 3253	BELIN MWB: 4212	BORRUAT FX: 4125

BORTHWICK NJ: 4434	CASTANHEIRA-DINIS A: 328, 2122, 2123, 3363	DAHLIN D: 460
BOSCH F: 4163	CASTEJON MA: 326 , 464	DAINTY C: 3325
BOSCH JI: 4431	CASTELO-BRANCO M: 243, 3442	DAINTY JC: 3447
BOSCHI A: 4122	CASTILLO A: 417, 2256	DALAL R: 3263
BOSSY-NOBS L: 229, 2346	CATALANO CC: 223 , 212, 436	DALIMIER E: 3447
BOURGES J-L: 352, 4114	CATERSON B: 209, 2332	DALKE C: 3212 , 3213
BOURNE WM: 2135	CEHAJIC: 230, 2347	DAMATO B: 1311, 3133
BOURS S: 2362	CELLINI M: 418	DANA RM: 364, 2131
BOYCHUK IM: 406 , 405	CERASE A: 486	DANDEKAR SS: 2465, 3322
BRADSTREET C: 410	CESAREN G: 3165	DARTT D: 215, 371, 2126
BRADU A: 3325	CEZAYIRLI E: 462	DASTUGUE B: 202, 2337
BRANDAO E: 2413	CHAING YM: 4454	DAVIES LN: 4364
BRAUTASET RL: 251	CHAKRAVARTHY U: 318	DAVIES VJ: 3214 , 3314, 3414
BREDEWOUD RA: 4362	CHANDRAMOHAN K: 315	DAYANIR V: 1341
BREMOND GIGNAC D: 4412	CHANG HR: 384	DE BENITO L: 369, 3453
BRETILLON L: 4441	CHANG JH: 2326	DE BRUIN DM: 3324
BREUER B: 310	CHAROENROOK V: 332, 2138	DE CLERCQ E: 1001
BRIAT B: 234	CHATEAU N: 253, 3258 , 3253	DE CRECCHIO G: 221
BRISCOE D: 3436	CHATR-ARYAMONTRI A: 3165	DE FRANCESCO S: 486 , 2431, 2435, 4333
BRISSE H: 2432	CHATZIBALIS T: 439 , 465, 3151	DE FRUTOS-BARAJA JM: 485 , 3331
BRON AJ: 366 , 209, 2234 , 2332	CHATZOULIS DZ: 336, 3156	DEGEER A: 4435
BRON AM: 214 , 335, 1322 , 2125 , 4441 , 3155, 3264	CHEMTOB S: 4348	DEGENRING RF: 312 , 301, 2467
BROOKS AC: 472	CHENG R: 2141	DE GRAAF P: 2434
BROUWER WH: 4362	CHEN-KUO-CHANG M: 3315	DE HOZ R: 207
BRUENECH JR: 2335	CHENTSOVA EV: 479, 4337	DE JONG PTVM: 2242, 2453
BRUZZICHESI D: 401, 409, 3127	CHERNYAK DA: 2155	DE KEIZER RJW: 484 , 477, 3431 , 3437
BRZHESKY VV: 383	CHEVROLLIER A: 3412	DE KOZAK Y: 2121
BUCH G: 331, 2137	CHIAMBARETTA F: 202, 2337	DELACROIX S: 3135
BUCHT C: 227, 2344	CHIANG MY: 421 , 422, 4451 , 4457 , 4452	DE LAEY JJ: 4463
BUGRA K: 2312	CHIQUET C: 445 , 214, 2124 , 3425 , 2125	DELAMERE NA: 2334
BUNIATYAN IY: 4455	CHISHOLM CM: 4364, 4365	DE LA PAZ M: 332, 2138
BURKE J: 3461	CHOMYAKOVA EN: 2152	DE LA VEGA C: 327
BURSELL SE: 3323	CHRISTMANN S: 301 , 2467	DELETTRE C: 3315
BURSTEDT MSI: 3364	CHRONI FC: 386	DELGADO E: 4241
BUSHUYEVA NN: 405	CHURCHILL AJ: 2414	DELLBY A: 348
BUSSCHER RB: 4362	CILLIERS H: 424	DEMBINSKA O: 4348
BUTTON NF: 362	CLARK HW: 2234	DENDALE R: 3132 , 2362, 2432, 3135
BYARD S: 419	CLARKE M: 3434	DENG W: 3152
BYSTRÖM B: 2331	CLERMONT A: 3323	DENIS P: 4353 , 2124
CACKETT P: 231	COCHEREAU I: 1323 , 2225	DEPEYRE C: 3315
CALDIRONI P: 379	COCKRUM P: 460	DESCHRYVER I: 2321 , 1321
CALIENNO R: 3352	COGNE M: 372	DESJARDINS L: 2362 , 2432 , 3135 , 4331
CAMPAGNA F: 401, 3127	COLEMAN AL: 2452	DE SMEDT S: 1353
CAMPBELL D: 2134	CORDES AK: 301, 312, 2467	DE SMET M: 1326 , 2224 , 2461 , 3325
CAMPILLO-FERNÁNDEZ A: 368	CORNILLE K: 3315	DE SOUZA-RAMALHO P: 4322 , 4323
CAMPOS EC: 418	CORNUT PL: 214, 2124, 2125	DETTRY-MOREL M: 466
CAMPOS S: 243	COSSET JM: 3132	DE VOOGD S: 2453
CAPOROSSI A: 210, 358, 441	COSTA N: 340, 3355	DE VRIES HH: 2162
CARBALLO-ALVAREZ J: 365	COSTANTE G: 409	DE WOLFF ROUENDAAL D: 477, 484, 3431, 3437
CARELLI V: 3311	COSTANTINO O: 4453	DHILLON B: 231
CARLSSON T: 467	COTERO J: 201, 2336	Diabetic Retinopathy Screening Team: 306
CARMELIET P: 2251, 4164	COURTOIS Y: 2315	DIABOLD Y: 215, 2126
CARNOVALE SCALZO G: 409 , 212, 222, 322, 401, 3127	CREE IA: 4434	DICK HB: 3343
CARTA A: 217	CREUZOT-GARCHER C: 214, 2124, 2125, 4441	DIEDEREN R: 3265
CARTA F: 217	CUCU R: 3325	DIELMAN C: 410
CARTER JG: 2414	CUENCA N: 208, 4348	DIETRICH T: 338, 356, 3158
CASAER P: 1354	CUMBERLAND PM: 2111	DIEUDONNE SC: 3265
CASANOVA C: 4348	CUNHA-VAZ J: 2464 , 3167 , 3463 , 2163, 2164, 2167, 3366	DIGHIERO PL: 234 , 334, 342, 3255 , 2132, 2235, 315a, 3251, 3354, 3357, 4111
CASEY JR: 242	CURRY S: 460	DI MATTEO F: 4153
CASPERS L: 410, 2323	CURSIEFEN C: 338, 356, 3351 , 4225 , 3158	DI NUZZO A: 221, 2165

- DOBRE GM: 3325
 DODI PL: **340, 3355**
 DOHM S: 301, 312, 2467
 DONG X: 227, 2144, 2344
 DONHAUSER N: 216
 DÖNMEZ A: 304
 DOOLEY JSG: 3152
 DORES J: 308
 DORFMAN A: 4348
 DORNER GT: 3332, 3421
 DOUGHTY MJ: 362
 DOWNES CS: 215, 2126
 DOZ F: 2362, 2432
 DREL VR: 4442
 DRILLAT P: 445, 3425
 DROUET M: 372
 DRUZDZ A: 4464
 DUA HS: **2233**
 DUARTE L: 3366
 DUKER JS: 3323
 DUMITRAS S: 481
 DUMOLLARD JM: 475
 DUNNE MCM: **4364**, 4365
 DZHEMILEVA L: 374, 2416
 EBRAERT H: **2323**
 ECKART R: 3324
 ECONOMIDES P: 439, 465
 ECONOMOU M: **4436**
 EDGAR DF: 4364, 4365
 EGEA MC: **428**, 320, 427
 EHLKEN C: 3143
 EKLUND A: 2254
 ELACHOURY G: 3315
 ELENA PP: 335, 3155
 EL-REMEZZY A: 4442
 EPERON S: **229, 2346**
 ESEN A: 304
 EWINGS P: 302, 3266
 EYSTEINSSON T: 448, 454, 4245, 4252, 4444
 FABER DJ: **3324**
 FABIANI L: 367, **2133**
 FAGERHOLM P: 348, 354, 355, **4211**
 FAGERUDD J: 307
 FALCAO-REIS F: 470, 2413
 FAN X: 2141
 FARIA P: **3442**
 FARKAS A: 323, 382, **4465**, 3362
 FASANELLA V: 4453
 FEKE GT: 4344
 FELEKIDIS A: 458
 FERNANDES AP: 473
 FERNANDES P: **440**
 FERNANDEZ I: 324
 FERNANDEZ-CRUZ A: 417, 2256
 FERNANDEZ DURANGO R: **417, 2256**
 FERRARA P: 367, 2133
 FERRE M: 3411
 FERREIRA A: 4341
 FERREIRA C: 2122, 2123
 FERRER B: 319, 457, 4447
 FERRERAS A: **429**, 425, 426, 432, 434, 435, **4154**, 4155
 FILIPEC M: 226, 349, 353
 FIOCCO R: 3164
 FIORE B: 211
 FISHMAN HA: 3263
 FISSON S: 2121
 FLAMMER J: 241, 451, 4254, 4343, 4345
 FLANAGAN DW: 305
 FLANAGAN J: 361
 FOLBERG R: 3335
 FONSECA J: 2161, 4341
 FORSBLOM C: 307
 FORTER R: 221, 2165
 FOSSARELLO M: 381
 FOULKS GN: 366
 FRAJDENBERG A: 375, 2417
 FRANKLIN V: **345**, 361, **3456**, 4213
 FRANSEN MF: 4433
 FRANZCO DM: 424
 FRANZCO GB: 424
 FRANZCO MC: 424
 FRASER S: 3215
 FRATTO MF: 223, 436
 FRAZER DG: 385, 3153
 FRENKEL S: **4332**, 3335, 4334
 FREZZOTTI P: **441, 2435**
 FRIC J: 226
 FROELICH CJ: 4435
 FRUSCHELLI M: **210**, 358, 486
 FRUTTIGER M: 3142
 FUCHSJÄGER-MAYRL G: 447, **3422**, 3424, 3427
 FUENTES I: 236, 369
 FUJIMOTO JG: 3323
 FUNK J: 423, 2354, 4458
 GABEL VP: 3444
 GAIN P: 333, 337, 475, 2139, 3157, 4214
 GAJECKA M: 375, 2417
 GALAND AJ: **3342**
 GALANTUOMO MS: 381
 GALE A: **4361**
 GALE RP: 228, 2345
 GALLUZZI P: 4333
 GAN L: 4211
 GANDHI JS: **213**
 GANDOLPHE C: 468
 GANDORFER A: 206
 GARCHER CREUZOT C: **335, 3155, 3264**
 GARCIA P: 3325
 GARCIA-ALVAREZ C: **480, 483**, 485, 3331
 GARCIA-BUENO B: 417, 2256
 GARCIA FEIJOO J: 417, 2256
 GARDINER T: 3153
 GARHOFER G: 446, 447, **3424**, 3332, 3421, 3422, 3426, 3427
 GARRAUD O: 475
 GARWEG JG: **2223**
 GARYFALLOS A: 439
 GAWLEY SD: **385**
 GAZZARD G: **2212**
 GEE H: 361
 GEISER MH: 445, 3425
 GEKELER F: 3444
 GHERGHEL D: 452, 4346
 GHYCZY-CARLBORG EAE: 2224
 GIAMBENE B: 2412
 GIANNOUSIS M: 471
 GICQUEL JJ: 234, 253, 334, 342, **2132, 2235, 3251, 3354, 4111**, 315a, 3255, 3357
 GIL R: 4456
 GILBERT C: 2243
 GIL-CAZORLA R: **236, 3451**
 GILES TG: **344, 3455**
 GILMARTIN B: **2113**, 2215
 GILMOUR DF: **231**
 GINI G: 2226
 GIRÃO H: 311
 GISLADOTTIR S: **448, 4245**
 GOES F Jr: **4411**
 GOMBERT JM: 334, 2235, 315a
 GOMES N: 2413
 GÓMEZ-RIBELLES JL: 368
 GOMEZ-ULLA F: 324
 GONCALVES ROSADO JL: **248**
 GONZALEZ F: 433
 GONZALEZ MARTINEZ M: **360**, 250, 255
 GONZALEZ-MEIJOME JM: 363, 440, 3445
 GOODALL E: 371
 GORBAN AI: 2157
 GORDON-BENNETT PSC: **305**
 GOS R: 459
 GOULAS A: 471
 GOULIA O: 225
 GOUREAU O: 3164
 GRABNER G: 332, 2138
 GRAF C: 3324
 GRAHAM JE: **215, 371, 2126, 3152**
 GRAMER E: 408
 GRAMER G: 408
 GRAW J: **2341**, 3212, 3213
 GRAY R: 302, 3266
 GREENWOOD J: 3142
 GREGORY M: **4432**
 GRIFFITH M: 4211
 GRIFFITHS G: 2134
 GRINBERG E: **374, 2416**
 GRISAN E: 373
 GROBUSCH MP: 218
 GROOP P-H: 307
 GROSS N: 376, 3216
 GRZYBOWSKI AE: **239, 3126, 4464**
 GUARESCHI M: 4153
 GÜERRI N: 320, 428
 GUERRI MONCLUS N: **427**
 GUEX-CROSIER Y: 229, 2346
 GUGGENHEIM JA: **2114**
 GUGLETA K: 451, 4345
 GUILLAUBEY A: 3264
 GUILLET V: 3412
 GUILLONNEAU X: 3164
 GULLBERG D: 2331
 GUMMEL KK: **383**
 GUNGOR K: **462**
 GURBAXANI A: **2326**

- GURNY R: 229, 2346
 GUTH C: 407
 GUTHOFF RF: 4311
 GUTIERREZ C: **420, 463**
 HABERAL N: 4242
 HADJISTILIANOUT: 441, 486, **2431, 4333**, 2435
 HAKA G: 210
 HALEY CS: 2114
 HALLBERG P: **2254**
 HALLDORSSON GH: 454, 4252, 4444
 HALLE M: 4342
 HALLERMALM K: **4435**
 HAMEL CP: 3315
 HAMILTON DL: 3154
 HAMMAR B: 348
 HAMMER M: 3321
 HAMMES HP: **4222**
 HAMMOND C: **2115**, 2111, 2243
 HANSEN LL: 376, 3143, 3216
 HARDARSON SH: **454, 4444**, 4252
 HARDER B: 2466
 HARDING J: **2142**
 HARDING S: 309, 318
 HARGITAI J: 323
 HARLOW JA: 3443
 HARRAD RA: 2211
 HARRIS A: **4255**, 2253, 2355, 4252, 4253
 HART-HOLDEN N: 4463
 HATHAWAY M: 3325
 HAUPT S: 216
 HAWLINA M: **1331, 4462**
 HAYASHI T: 2413
 HAYES V: 3152
 HAYWARD JM: 228, 2345
 HEEG GP: 431, 4157
 HEEGAARD S: **3433**
 HEGDE K: **2143**
 HEIJL A: 4151
 HEILIGENHAUS A: 2325
 HEIN L: 376, 3216
 HEINRICH SP: **3441**
 HEINZ C: 2325
 HEISTERKAMP A: **3262**
 HEITMAR R: **452, 4346**
 HELMANOVÁ I: 232
 HENDRIKSE F: 3265
 HENLEY CE: **476**, 361
 HENRIQUES J: 328, 3363
 HERNANDEZ VERDEJO JL: **319, 457**, 236, **4447**, 3451
 HICKS C: **4113**
 HICKS D: **2312**
 HIETALA K: 307
 HIGGINS GT: **309**
 HILD M: 3261
 HIROSE T: 4344
 HITZL W: 332, 2138
 HODGE DO: 2135
 HOFMAN A: 2453
 HOLAN V: **349**, 226
 HOLDER GE: **1332, 1334, 2261, 4263, 4466**, 3361
 HOLLINS AJ: **3414**, 3214, 3314
 HOLLÓ G: **2451, 4256**
 HOLMES JM: **3123**
 HOLOPAINEN J: 313
 HOLZ FG: 3112, 3145
 HONRUBIA FM: 425, 426, 427, 428, 429, 432, 434, 435, 4154, 4155
 HOPKINSON A: 2233
 HORNOF M: 449, 4246
 HOUA N: 410
 HOWARD M: 2211
 HRABEC E: 459
 HRABÉ DE ANGELIS M: 3212
 HUL: 2454
 HUA J: **376, 3216**
 HUBERT I: 3264
 HUEMER K-H: 3422
 HUGHES C: 209, 2332
 HUHTALA A: 474
 HUIE P: 3263
 HULEWICZ A: 239
 HUNGERFORD JL: 3336
 HUNT DM: 4466
 HUNTBACH JA: **370**
 IHLOFF AK: 2466
 IKRAM K: 357
 IMHOF S: **2434**
 IMMONEN I: 313, **3115**
 IMPAGNATIELLO F: 456, 4446
 IOANNIDES A: 2411
 IOMDINA EN: 256, **2156**
 IPINAZAR UNDURRAGA M: **460**
 IVANITSKAYA EV: 406
 JACKSON AJ: 245, 246, 3153
 JAGER MJ: **4433**, 4434, 4435
 JAMES O: 472
 JANOWCHA A: 238, 407
 JANSONIUS NM: 431, 2453, 4157
 JARKÖ C: 251
 JARVIS S: 2141
 JASHEYAW D: 460
 JAVALOYES MORENO B: **250**, 255, 360
 JAYAPRAKASAM A: 421, 4457
 JAZAYERI F: 325
 JEANNY JC: 352
 JENKINS S: 2465, 3322, 4466
 JEON SL: **384**
 JIANG JX: **3242**
 JIRSOVA K: 226, 353
 JO N: 3121
 JOCHMANN C: 310
 JOELSSON SR: 454, 4444
 JOFFRE C: 335, 3155, 4441
 JOLY S: 4348
 JONAS JB: **314**, 437, **2454**, 2257, 2466, 3144
 JONUSCHEIT S: **362**
 JORGE J: **254**, 363, 440, **3445**
 JOURNÉE-DE KORVER H: **3134**
 JUKLOVA K: **353**
 JULIUS U: 4442
 JURKLIES B: 4336
 KAARNIRANTA K: **313**, 203, 204, 415, 474, **3113**
 KAEMMERER E: 3112
 KAIMBO WA KAIMBO D: **247**
 KAKAR M: 227, 2144, 2344
 KALATHAS T: 3151
 KALESNYKAS G: **416**, 415
 KALICKMAN I: 3335
 KALLIO J: 313
 KAMPIK A: 206, 3141, 4244
 KAMPOURIDIS S: 410
 KAMPETER BA: **437, 2257**
 KARAASLAN P: 304
 KARABATSAS CH: 336, 3156
 KARAMITSOS A: 3151
 KARAMPATAKIS V: **465, 471**, 225, 439, 3151
 KARIN M: **3241**
 KARL K: **446, 3426**, 3422
 KARLSSON JO: 467, **2333**
 KARLSSON RA: 454, 4252, 4444
 KARVONEN M: 313
 KÄSNÄNEN H: 416
 KASPRZAK H: 343, 3454
 KATAMAY R: 451, 4254, 4345
 KAUP M: 2252
 KAWASAKI A: **4124**
 KAZLAUSKAS A: 364, 2131
 KERN S: 3144
 KERNT M: 4244
 KERR BC: 209, 2332
 KERR J: 361
 KERR-MUIR M: 228, 2345
 KESSELS A: 3265
 KESTELYN P: **1321**, 2321, 4463
 KHETERPAL S: 386
 KHURIEVA EL: 3261
 KHUSNUTDINOVA E: 374, 2416
 KIDRON D: 3436
 KIESLING R: 4435
 KIJLSTRA A: 3265
 KILBASANI M: 471
 KIM CY: 414
 KIM R: 315
 KIM SG: 414
 KIRCHER K: 3421
 KITTAN N: 216
 KIVELÄ T: **1311, 3131**, 3435
 KJELLEVOLD HAUGEN IB: 2335
 KLATZMANN D: 2121
 KLAVER CCW: **2242**
 KLUETER H: 3144
 KNEZY K: 323
 KNOP E: **2231**, 2232
 KNOP N: **2232**, 2231
 KOCHKOROV A: **451, 4345**
 KODJIKIAN L: **1324, 2222**, 2223
 KOENIG K: 3261
 KOKKINOU D: 458
 KOLEDA P: 238
 KOLODJASCHNA J: 3423
 KOMOROWSKI J: 459
 KONTTINEN L: 348

KOOIJMAN AC: 4362	LENGYEL I: 2245	MACDONALD IM: 242
KOPITZ J: 3112	LEONETTI P: 418	MACERI C: 3122
KORN K: 216	LEROUY BP: 1333, 4262, 4463	MACKENSEN F: 2325
KOSHAL RAM B: 315	LESCH B: 323 , 382, 3362	Macugen Diabetic Retinopathy Study Group: 2464
KOSHITZ IN: 2157 , 2153	LESKE DA: 3123	Macugen Retinal Vein Occlusion Study Group: 2463
KÓTHY P: 2451	LEUNG I: 2465	MAESTRONI L: 321, 2462
KOTLIAR KE: 211, 2151, 4342 , 2153, 2157, 3446	LEVILLAIN PM: 342, 3354, 3357	MAGNUSSON GCB: 235
KOUFAKIS DI: 336, 3156	LEVITSKAYA J: 4435	MAHOMED A: 450, 4247
KOULIKOVSKA M: 355	LEVITSKY V: 4435	MAIA-LOPES S: 243
KOULU M: 313	LEVY-CLARKE G: 2324	MAIER M: 211
KOZEIS N: 458, 3124	LEVY-GABRIEL C: 2362, 2432, 3135, 4331	MAIMONE PE: 379
KRASNITSKAYA S: 2153	LEWANDOWSKA AB: 459	MAJUMDAR D: 3335
KRAUSE MH: 3261	LEZA JC: 417, 2256	MALANDRINI A: 358
KREISSIG I: 2466	LI DW: 3244	MALIK ANJ: 412, 2456, 3336
KRIAUCIUNIENE L: 252	LI XQ: 330, 331, 2136, 2137	MALLOCI C: 381
KROHNE T: 3112	LI Y: 314	MALTHIERY Y: 3411, 3412
KRUEGER RR: 3262	LI Z: 1355, 2251	MALVITTE L: 3264
KRULOVA M: 349	LIBERALI T: 3352	MANDRAVELI K: 471
KRUSE FE: 338, 356, 3158, 3351, 4313	LIBONDIT T: 2466	MANIK KS: 213
KSANDER BR: 4431 , 4432	LIEBERT W: 4464	MANISSOLLE C: 475
KUCHLER F: 445, 3425	LIEKFELD A: 218	MANIVANNAN A: 2166
KUEHL S: 3144	LIEM A: 3265	MANN A: 346, 341, 3457 , 3356
KUHN F: 2226	LIEW M: 2243	MANOJ S: 315
KUPPERMANN BD: 3462	LIGHTMAN S: 2326	MANTHOS AK: 336, 3156
KURZ S: 3343	LINDBOHM N: 348	MARCEAU G: 202, 2337
KUTTENKEULER C: 3444	LINDEN C: 2254	MARCOS S: 2443, 3256
KUUSISTO E: 204	LINDSKOOG PETTERSSON A: 251	MARDIN CY: 430, 4156
KVANTA A: 4162	LINDSTRÖM J: 3365	MARIGO V: 3164
KYTÖ JP: 307	LINDSTRÖM-KARJALAINEN M: 307	MARINA C: 319, 4456
LABONIA AF: 409	LITTLE BC: 3344	MARKLUND SL: 455, 4445
LACHAPELLE J: 237	LIU C: 3341, 4112 , 4213	MARMOR MF: 3263
LACHAPELLE P: 4348	LIU J: 3244	MARQUES C: 4255
LAEMMER R: 430, 4156	LIU JJ: 3323	MARQUES CJ: 2413
LAFAUT BA: 4463	LIU M: 3243	MARQUES-NEVES C: 4241
LAFONTAINE PO: 214, 335, 2124, 2125, 3155, 3264	LIU W: 4211	MARQUET P: 337, 3157
LA HEIJ EC: 3265	LJUBIMOVA DY: 438	MARSHAK-ROTHSTEIN A: 4432
LAM BL: 4461	LLOYD AW: 4213	MARSHALL J: 2243
LAMBIASE A: 3353	LO JP: 3452	MARTIN G: 376, 3143, 3216
LAMBLEY RG: 302, 3266	LOCKWOOD A: 422, 421, 4452, 4451, 4454, 4457	MARTIN L: 244, 1342
LAMOUREUX E: 424	LODI R: 3413	MARTINEZ A: 4325
LANG C: 366	LOEFFLER KU: 3145	MARTINEZ DE LA CASA JM: 417, 2256
LANGE CAK: 376, 3143 , 3216	LOEW U: 3261	MARTINEZ-GARCIA A: 461, 2455
LANGENBUCHER A: 2352	LÖFGREN S: 227, 2144, 2344	MARTINEZ-GARCIA MC: 2133, 3353
LANGMAN ME: 334, 342, 2236, 315a, 3357	LOFTSSON T: 4443	MARTINEZ-NAVARRETE G: 208
LANZINI M: 4453 , 3352	LOGAN NS: 2215 , 2113	MARTONE G: 210
LANZL I: 211, 3446 , 2151, 4342	LOISEAU D: 3412	MARTUS P: 430, 4156
LARROSA JM: 425, 426, 429, 432, 434, 435, 4154, 4155	LOMURNO L: 441	MASTROPASQUA L: 4312 , 3352, 4453
LASUDRY J: 4131	LONCASTER J: 4231	MATHEW M: 2233
LAUTEBACH S: 2354	LONGO I: 441	MAYORAL F: 426, 429, 435, 4155
LAYER PG: 2314	LOPES D: 4341	MAZZAROLO M: 379
LAZUK AV: 256	LOPEZ GIL N: 253, 3252	MCCLUSKEY P: 2326
LEACH C: 4231	LOPEZ-LARA F: 480, 485, 3331	MCGIMPSEY S: 215, 2126
LEAL SM: 375, 2417	LOPEZ PENA MJ: 425	MCINTOSH I: 378
LEBRETON P: 233	LORENZ B: 2263, 2363	MCKERRAL M: 237
LEBUISSON DA: 3253	LÖSTER J: 2341	MCMEEL JW: 4344
LEE GA: 4451	LUBATSCHOWSKI H: 3262	MEEK KM: 201, 209, 377, 2332, 2336, 3217
LEHOANG P: 1325, 2121	LUGO F: 469	MEIRELES A: 308
LEITE-MOREIRA AF: 470	LUMBROSO LEROUIC L: 2362, 2432, 3135, 4331	MELIEF CJM: 4433
LELUJK VG: 478, 3438	LUND RD: 208	MELIS-DANKERS BJM: 4362
LENAERS G: 3315	LYUBIMOV GA: 2154	MELLERIO J: 2243
LENG T: 3263	MA KT: 414	MELONI I: 441

- MENCHINI U: 380, 2412
 MENDEZ MC: 480, 483, **3432**
 MENICACCI F: 486, 2435, 4333
 MERAYO-LLOVES JM: 367, **4414**, 2133, 3353
 MERIGAN W: **2422**
 MERINO D: 3325
 MERMOUD A: 465
 MESEGUER-DUENAS JM: 368
 MESIANI MAZZACUVA FMM: 212, 223, 436
 MESNIL M: 342, 3354, 3357
 MESSMER EM: 211, **4315**
 MESTRES P: 3261
 MEYER L: 227, 2144, 2344
 MICHAEL R: **332**, **2138**, 2446
 MICHAELIDES M: 4466
 MICHALEK J: 4152
 MICHAUD JE: **472**
 MICHEL O: 2323
 MICHELS BPJ: 2162
 MIDENA E: **3136**, 3333
 MIETTINEN R: 204, 415
 MIGLIOR S: 321, **4153**, 2462
 MIGUEL L: **308**
 MILANO G: **3122**
 MILEA D: **4123**
 MILIN S: 342, 3354, 3357
 MILLER L: 2212
 MINASSIAN DC: 316
 MIRANDA M: 2122, 2123
 MIRON I: 481
 MIRTSOU V: 471
 MISKOWIAK B: 4464
 MISSOTTEN GS: **477**, **3326**, **3437**
 MISSOTTEN L: 247
 MISSOTTEN T: **220**, **2322**
 MITTICA V: 441
 MODY VC: **227**, **2344**, 2144
 MOHNEY BG: 3123
 MOLL A: 2434
 MOLNAR FE: **3263**
 MONNIER V: **2141**
 MONTEIRO-GRILLO M: **4341**, 2122, 2123
 MONTERO J: 327, 469, **4243**
 MONTES MICO R: **3257**
 MOORE AT: **2001**, 4463, 4466
 MOORE CBT: 215, 371, 2126, 3152, 3153
 MOORE JE: **371**, 215, 2126, 3152, 3153
 MOORE JE: 215, 2126, 3152
 MORA A: **2161**, 4341
 MORGAN IG: **2213**, 2214
 MORGAN LH: 213, 357
 MOSCATO B: 221, 2165
 MOSS S: 3142
 MOTOC I: 481
 MOTOLESE P: 441
 MOTULSKY E: **1351**
 MOTYKA B: 4435
 MOURIAUX F: **4233**
 MOUTRAY TN: **245**, 246
 MRUGACZ M: **350**
 MUINOS Y: 3331
 MULDREW KA: **318**
 MULLER A: 3315
 MÜLLER LJ: **4313**
 MULLINS RF: **3114**
 MUNIER F: **2433**
 MUÑOZ MA: **433**
 MUÑOZ MF: 3331
 MUQIT MMK: **4232**
 MURA M: 2224
 MURRAY C: 386
 MUSHTAQ B: 329
 MUSKENS RPHM: **431**, **2453**, **4157**
 MUTHIG V: 376, 3216
 MYCHASKIWA MA: **443**, 413, 442, 444, 2457
 NAKAZAWA T: 3121
 NAMPERUMALSAMY P: 315
 NASCIMENTO J: 328, 3363
 NASCIMENTO SMC: 248
 NATSIS K: 225, 439, 465
 NAVARRE R: 2235, 4111
 NEIRA W: **348**
 NEPP J: **3125**
 NESHER R: 3436
 NEUBAUER AS: 4244
 NEUBURGER M: 2354
 NEUGEBAUER M: 378
 NEUWIRTH A: **226**
 NEWSOM W: 305
 NEZZAR H: 202, 2337
 NG YS: 3121
 NGUYEN KHOA JL: 253, **3253**, 3258
 NICOLAISSEN B: 351
 NIETO A: **365**
 NIITTYKOSKI M: **415**
 NILSSON M: **244**
 NISHIJIMA K: 3121
 NOISSER T: 3324
 NORTHSTONE K: 2211
 NOWAK MS: 459
 NTAMPOS K: **225**, 439, 465
 NUBILE M: **3352**, **4314**, 4453
 NUNES S: **3366**, 2163
 NURISPAHIC A: 3446
 NUSSENBLATT RB: 2324
 NYSTRÖM A: 235
 OBRENOVICH M: 2141
 OBROSOVA IG: **4442**
 ODAT T: 213
 O'DONNELL C: 3254
 OKSÅLA E: **474**
 OLICHON A: 3315
 OLIVEIRA FERREIRA JV: **311**
 OLOFSSON EM: **455**, **4445**
 ONDERKA J: 338, 356, 3158
 OOI KGJ: 3361
 ORGUL S: 241, 451, **4254**, 4343, 4345
 ORTEGA M: 3453
 ORTWERTH B: 2141
 OSBORNE NN: **2241**
 ØSTERGAARD J: 3433
 OSTRAND-ROSENBERG S: 4431
 OTASEVIC L: 331, 2137
 OTO S: 4242
 OWEN CG: 2215
 OWEN GR: 472
 OWENS H: **3452**
 OZBILGIN K: 462
 OZDEN R: **1343**
 OZKAN SB: **3221**
 PAAVILAINEN V: 4132
 PABLO LE: **320**, 427, 428, 4154
 PAC L: 303, 3267
 PACHE M: 423, **2351**, 4458
 PAIMELA T: **203**
 PAJARIN AB: 429, 4154
 PALMOWSKI-WOLFE AM: 241
 PALOMBI K: 2124
 PALOMO-ALVAREZ C: 365
 PAMER Z: 323
 PANDIT RJ: 219, 224
 PANTELEEEVA OG: 240
 PAOLA A: 401, 409, 3127
 PAPANIKOLAOU T: 471
 PAPP A: 323
 PARAFITA MA: 254, 363, 440, 3445
 PARDATSCHER KP: 212
 PARISE S: **222**, 322, 401, 3127
 PARK CY: 384
 PARROZZANI R: **3333**
 PASQUALE LR: 4344
 PASSEMARD M: 214, 335, 2125, 3155
 PASSERINI I: 380, 2412
 PASTOR S: **339**, **368**, **3159**
 PASTOR JIMENO JC: 324
 PATEL S: **2463**, 2135
 PATTON WP: 318
 PAULEIKHOFF DP: 2244
 PAUNKSNIS A: 252
 PAYET O: 3315
 PECHINOT A: 214, 2125
 PECKHAM CS: 2111
 PEDRO J: 3325
 PEDROSA-DOMELLÖF F: 2331
 PE'ER J: **4334**, 3335, 4332
 PEGO P: **4324**
 PELTONEN M: 3365
 PENAS S: 2413
 PENDINO V: **322**, 222, 401, 3127
 PEOCH M: 475
 PEREIRA P: 205, 311
 PEREIRA DA SILVA A: **4323**, 4322
 PEREZ S: 427, 428
 PESCE GP: **212**, 223, 436
 PESONEN U: 313
 PETERSEN A: **467**, 2333
 PETO T: **306**, 318, **2465**, **3322**
 PETROPOULOS IK: 229, 2346
 PETZOLD A: **4363**, 4365
 PICCIRILLO V: **221**, **2165**
 PIECHOTA MJ: 3214, 3314, 3414
 PILECKI W: **238**, 407
 PINELLO L: **379**

- PINERO A: 324
PINILLA I: **208**, 242, 4348
PINNA A: **217**
PINSKY PM: 2155
PITZ S: **2411**
PIZZAMIGLIO-MARTIN C: **369**, **3453**, 4456
PLAKE K: 413, 2457
PLANCHER C: 3135, 4331
PLANGE N: 2252
PLANT G: **3223**, **3415**, **4366**, 4363, 4365
PLEYER U: **331**, 330, **2137**, 2136
POCH O: **3161**
PODFIGURNA-MUSIALAK M: 375, 2417
PODOLEANU A: **3325**
PODKOCHY A: **354**
POKORNA K: 349
POLAK ME: **4434**
POLO V: 425, 426, 429, 432, 434, 435, 4154, 4155
POLSKA E: **3423**, 3422
PONG-WONG R: 2114
PORCU F: 217
POU MA: 3412
POULIQUEN P: 335, 3155
POURJAVAN S: **466**
POURNARAS JAC: **3334**
POWLING A: 306
PRADA J: **218**
PRADES JM: 475
PRAUSE JU: 3433
PREISING MN: **2262**
PROENCA H: **2122**
PROENCA R: 473, 3353
PUECH B: 4463
PUELL MC: 365, 433
PUEYO V: **432**, 426, 429, 434, 4154
PUK O: **3213**, 3212
PURVIN V: **4121**
PYROCHTOVA S: 2343
QIAN J: 3211
QUEIROS A: **363**, 440, 3445
QUINTAO T: 328
RADHAKRISHNAN H: **3254**
RAHI JS: **2111**
RAHMAN I: 2233
RAJA V: **315**, **378**
RAMIREZ AI: **207**
RAMIREZ JM: 207
RAMOS P: **473**
RAMSTAD H: 351
RANGER-ROGEZ S: 337, 3157
RANKIN SJA: 385
RANTALA J: 415
RAO NA: **2423**, **3002**
RAO V: 221, 2165
RAPTOU E: 3151
RASUL N: **359**
RAUSCHER FG: **4365**, 4363, 4364
RECHICHI MR: **436**, 212, 223
REEVES B: 318
REHUREK J: 232, 2343
REINHARD G: 4254, 4343
REIS A: 243
REITNER A: 3421
REME C: **4001**
REMKY A: **2252**
RENEKER L: 2141
RENIER C: **2255**
RENIERI A: 441
RESCH H: 446, **3332**, 3421, 3422, 3426
REYNIER P: **3412**, 3315, 3411
RICHTER-MÜKSCH S: 3125
RICKENBACHER I: 4343
RIEMANN I: 3261
RIGAL D: 202, 2337
RIGAL SASTOURNE JC: **233**
RILEY A: 309
RITTER T: 330, 2136
RIVA CE: **453**, **4347**
ROBERT PY: **337**, **372**, 475, **3157**
ROBERTSON SM: 472
ROBIN AL: **4351**
ROBINSON GS: 3121
ROBLOT P: 334, 315a
ROBSON AG: 3361, 4466
ROCHA I: 4241
ROCHA DE SOUSA A: **470**, **2413**
ROCHETTE L: 4441
RODRIGUEZ A: 339, 3159
RODRIGUEZ-CARMONA ML: 3443
ROGERS JA: 3325
ROJAS B: 207
ROJAS J: 324
ROLANDO M: **4413**
ROLLING F: **2361**
ROMAN JM: 457, 4447
ROMANET JP: 214, 445, 2124, 2125, 3425
RÖNKÖ S: 474
ROSA CC: 3325
ROSE KA: **2214**, 2213
ROSEN E: 3436
ROSEN R: 221, 2165, 3325
ROSENGÅRD-BÄRLUND M: 307
ROSNER M: **4133**
ROSS G: **347**, **3458**
ROTHERMEL A: 2314
ROTONDO E: 401, 3127
ROUBEROL F: 214, 2124, 2125
ROUSSANE MC: 337, 3157
ROVATI L: 453, 4347
ROY A: 460
RUDNICKA AR: 2215
RUEDA J: 4243
RUGGERI A: **373**
RUIZ E: 207
RUIZ-COLECHA J: 339, 3159
RUIZ MORENO JM: **327**, **469**, 368, 4243
RULLI E: 4153
RUPRECHT KW: 3261
RUTISHAUSER-ARNOLD Y: 2465
RYABTSEVA AA: **2152**
RYDZANICZ MA: **375**, **2417**
RYHÄNEN T: **204**, 203
SAAKYAN SV: **479**, 478, **4337**, 3438
SAARI KM: **4132**
SAAVEDRA J: 404
SACHS H: 3444
SAFF R: 4432
SAILER H: 3444
SAKELLARIS D: **1357**
SAKKAS LI: 336, 3156
SALACZ G: 323
SALAZAR JJ: 207
SALES-LUIS J: 4241
SALMINEN A: 203, 204, 415
SALOMON B: 2121
SALOMON E: 238, 407
SAMANIDOU E: 225
SANCHEZ A: 428
SANCHEZ JM: 236
SANCHEZ-CANO A: 320
SANCHEZ CHICHARRO D: **324**, **482**
SANCHEZ-MIGALLON MC: **403**, 249, 250, 402
SANCHEZ-PINA J: 3453
SANCHEZ-SALORIO M: 461, 2455
SANDANSHIV P: 378
SANDERS M: 460
SANDGREEN O: 3364
SANDHU S: 224, 3434
SANTOS J: 483
SANTOS T: **2164**, 2163, 2167
SANZ JC: 433
SAORNIL MA: 480, 482, 483, 485, **3331**, 3432
SAPIN V: **202**, **2337**
SARAIVA J: 470
SARASA JL: 3432
SARBU L: 481
SARFARAZI M: **2444**
SASTRE X: 2362, 2432
SAUVAGE FL: 337, 3157
SAUVE Y: **242**, 208
SAVASTANO MC: 221, 2165
SAVASTANO S: 221, 2165
SAVOLDELLI M: 352, 468
SAVY O: 445, 3425
SAW SM: **2112**, 2114, 2212
SAWITZKI B: 330, 2136
SCARMOUTSOS F: 309
SCHAFFER-YOUNG J: 257
SCHALENBOURG A: **4335**, 3334
SCHARGUS M: **408**
SCHILLING R: 2151
SCHLICHTENBREDE F: 2466
SCHLICKEISER S: **330**, 331, **2136**, 2137
SCHLINGEMANN RO: **4161**, **4224**, 2162
SCHMERMER C: 306
SCHMETTERER L: 446, 447, **4251**, 3332, 3421, 3422, 3423, 3424, 3426, 3427
SCHMIIDT B: 216
SCHMIIDT-ERFURTH U: **4165**
SCHMIDT-TRUCKSAS A: 4342
SCHOLL HP: 3162
SCHRAERMEYER U: 458
SCHRAGE NF: 301, 312, 2467

- SCHROETER J: 373
 SCHUMANN RG: **206**
 SCHUTT F: 3112
 SCHWARTZ GF: **413, 2457**
 SCHWEITZER D: **3321**
 SCHWEITZER F: 3321
 SCORCIA G: 222, 223, 322, 401, 409, 3127
 SCORCIA GB: 222, 322, 401, 409, 3127
 SCORCIA V: 222, 322, 409
 SCORRANO L: **3312**
 SCOTT A: **3142**
 SEIDOVA SFG: **240**
 SEITZ B: 3261
 SEKI K: 4435
 SEN HN: **2324**
 SEONG GJ: 414
 SERGUSHEV SG: 2152
 SERRA U: 3122
 SHAH P: 421, 422, 2215, 4451, 4452, 4454, 4457
 SHAH S: 3153
 SHAHIDULLAH M: 2334
 SHAKER M.H.DUHAIR MH: 405
 SHAM P: 2111
 SHAMSHINOVA AM: **411, 240**
 SHARMA A: 3153
 SHEPPARD JPB: **377, 3217**
 SHERIDAN L: 4231
 SHIMA DT: **3121**
 SHIN J: 4442
 SHUKLA D: 315
 SIABANOPOLOU M: 3151
 SICARD P: 4441
 SIDDIQUI S: **228, 2345**
 SIEGAL T: 4332
 SIESKY B: 2253, 4255
 SIEVING P: **257, 258, 1361**
 SIEVING PA: **2002**
 SIFFROI-FERNANDEZ S: 2312
 SIGURDSSON SB: 448, 4245
 SII F: 421, 422, 4451, 4452, 4454, 4457
 SILVA E: 243
 SILVA MF: 243, 3442
 SILVA BELTRÁN B: **2123**
 SILVA-CARVALHO L: 4241
 SINES D: 4255
 SINGH KD: 2113
 SJÖSTRAND JB: **235**
 SKINNER KC: **386**
 SKORKOVSKA S: **4152**
 SKRIAPA-MANTA A: 3151
 SLETTEDAL JK: **351**
 SMITH AF: **316**
 SMITH JA: 366
 SMOLNIKOV BA: 2157
 SOBIESZCZANSKA M: **407, 238**
 SOBRADO CALVO P: **255**, 249, 250, 360, 402, 403
 SÖDERBERG PG: 227, **2144, 2441**, 2344
 SODI A: **380, 2412**, 2411
 SOLEBO L: 228, 2345
 SOMANATHAN S: **329**
 SOMFAI GM: 323, 382, 3362
- SORO MARTINEZ MI: **249**, 250, 402, 403
 SOUKKARIEH C: 3315
 SOWDEN J: 3215
 SPANDAU UHM: 2466
 SPARROW JM: 2211
 SPIEERS W: **4261**
 SPINELLI A: 322
 SPRUIJT L: 2415
 SRINIVASAN VJ: **3323**
 STACHS O: **4311**
 STALMANS I: **2253, 2355, 4164, 4253, 4257**, 2251, 2255
 STAMBOLIAN D: 381
 STANA CS: 212
 STANGOS N: 465
 STEFANSSON E: 454, **4252, 4443**, 4444
 STENEVI U: 343, 3454
 STEWART JA: **442, 443, 444**
 STEWART WC: **444**, 442, 443
 STRAUSS R: 206
 STRICKER BHC: 2453
 SUMMANEN PA: 307, **3365**
 SUNDER-PLASSMANN G: 2411
 SVETLOVA OV: 2152, 2153, 2157
 SWAROOPI A: 2312
 SWEENEY E: 378
 SWIATEK-DE LANGE M: 3165
 SYNEK S: **303, 3267**
 SYNKOVA M: 303, 3267
 SZABO V: 323
 SZCZESNA DH: **343, 3454**
 TABERNERO J: 2442
 TAKEUCHI T: 2413
 TAVANDZI U: 349
 TEJERINA T: 207
 TEMPRANO J: 332, 2138
 TERADA C: 2121
 TERÄSVIRTA M: 313, 416
 TERVO T: 348, 4313
 TEUS MA: 236, 319, 326, 369, 420, 457, 463, 464, **4456**, 3451, 3453, 4447
 THOMPSON JA: 4431
 THURET G: **333, 475, 337, 2139, 4214**, 2124, 3157
 TIFFANY JM: 366
 TIGHE BJ: **361**, 341, 345, 346, 347, 359, 450, 476, 2134, 3356, 3456, 3457, 3458, 4213, 4247
 TISO C: 3122
 TODOROVA MG: **241**
 TOES REM: 4433
 TOMBRAN-TINK J: **2311**, 2313
 TONINI M: 445, 3425
 TOPOUZIS F: **4352**, 2452
 TOROPYGIN SG: **3261**
 TORRES J: 3451
 TORRICELLI F: 380, 2412
 TORRIGLIA A: 468
 TOSI GM: 358
 TÓTH M: 2451
 TRAVERSI C: 210
 TRAWICK D: 460
 TRIESCHMANN MBT: **2244**
- TRIVINO A: 207
 TSALOUMAS MD: 329
 TUFAN H: 4242
 TUGLU IH: 462
 TUISKU I: 348
 TUOMILEHTO J: 3365
 UEFFING M: **3165**
 UFFER S: 2433, 4335
 UNDERWOOD GJ: 4365
 URTTI A: 449, 4246
 USAI D: 217
 UUSITALO H: 203, 204, 313, 415, 416, 449, 474, **3111**, 4246
 UUSITUPA M: 3365
 VABRE L: 3258
 VACLAVIK V: **3361**
 VAIDEANU D: **3215**
 VALAMANESH F: **468**
 VALLE ML: 480, 483
 VAMOS R: **382, 323**
 VAN BELLINGHEN V: 2253
 VAN DAEL PL: 220, 2322
 VAN DELDEN: 4362
 VANDENESCH F: 2124
 VAN DER BLIEK A: **3313**
 VAN DER HEIDE DT: 2155
 VAN DER LOOS T: 220, 2322
 VANDERVOORT J: **1352**
 VAN DE VEIRE S: **1356**
 VAN DE VEL I: 2251
 VAN HAGEN PM: 220, 2322
 VAN KUIJK E: **2243**
 VAN LAAR JAM: 220, 2322
 VAN LEEUWEN R: 2242
 VAN LEEUWEN TG: 3324
 VAN MIERLO GJD: 4433
 VAN VELTHOVEN ME: 2162, 3324, 3325
 VARANO L: **401, 222, 322, 3127**
 VARGHA P: 2451
 VARMA S: 2143
 VAROL T: 462
 VARSANYI B: 323, **3362**
 VEHANEN K: **449, 4246**
 VELARDE J: 201, 2336
 VELUSAMI P: **224, 3434**
 VERBRAAK FD: **2162**, 3324
 VERGELY C: 4441
 VERNY C: 3411, 3412
 VETRINI F: 3164
 VIAU S: 335, 3155
 VIDAL LOPEZ J: **402**, 249, 250, 255, 360, 403
 VIDAL LOPEZ M: 4243
 VIDE ESCADA EICKMANN A: **328, 3363**
 VIEIRA P: 2161, 2166, 4341
 VIESTENZ A: **2353**
 VIITALA R: **4213**
 VILLA-COLLAR C: 363
 VILLAFRUELA I: **404**
 VILSER W: 2151, 4342
 VINH V: 445, 3425
 VOGEL A: 3145

- VOLK HD: 330, 2136
VOLPERT K: 2314
VON WENDT G: 3365
VOSSMERBAEUMER U: 3144
VOTRUBA M: 377, **3314**, 3214, 3217, 3414
VUJOSEVIC S: 3333
WANG J: 227, 2144, 2344
WANG Y: 2454, 2454
WEARNE IM: 230, 2347
WEBER A: 2252, 2341
WEBSTER AR: 2111, 2465, 3361, 4466
WEFERS BETTINK - REMEIJER M: **2415**
WEIGERT G: 446, 447, **3421**, 3422, 3426, 3427
WEISS R: 3325
WELGE-LUSSEN U: 206, **4244**, 3141
WENG J: 3243
WIEDEMANN P: 310
WILHELM B: 3444
WILKE R: 3162, 3444
WILLERMAIN F: **410**, 2323
WILLIAMS C: **2211**, 2212
WILLIAMS EE: 3154
WILLIAMS MA: **246**, 245, 385
WILLIAMS TA: 421, 422, **4454**, 4451, 4452, 4457
WILLOUGHBY CE: 385
WILMSMEYER S: 423, 4458
WITTEMAN JC: 2453
WITVLIET J: 4362
WITZENMANN T: 437, 2257
WOJTKOWSKI MD: 3323
WOLFF J: 3142
WOLFFSOHN J: 361
WOLFS RCW: 2453
WOLZT M: 3424
WONG E: 3452
WOO KI: 384
WOOD TO: 3154
WORSTMANN T: 370
WRIDE NK: **219**
WU H: 4466
WYSOCKA J: 350
XU J: 3152
XU L: 314, 2454
XU W: 4442
YANG H: 2454
YARDLEY J: 4463
YELLACHICH D: 3263
YEOM HY: **414**
YEUNG I: 3153
YGGE J: 383, **3222**
YILMAZ G: 304, 4242
YU AL: 206, **3141**, 4244
YU F: 2452
YU SX: 3242
YU X: 3211
YUE B: 202, 2337
YUEN L: 412, 2456
YUNG CW: 4255
ZACK DJ: 2421, 3211
ZAHN G: 3145
ZAJICOVA A: 349